Nanoflagellates of Southern Tasmanian Waters: Taxonomy, Toxicology and Distribution

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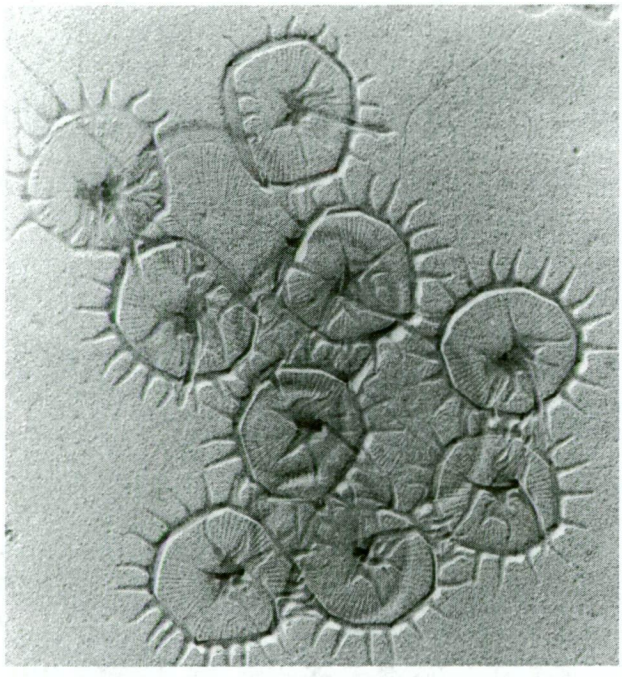

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ABSTRACT

A taxonomic survey of scale-bearing nanoflagellate algae from southern Tasmanian coastal waters was undertaken. Observations were made on 52 samples collected from 21 different sites (June 1994 - 1995) and resulting enrichment cultures. Scale morphology was examined using transmission electron microscopy.

Over 70 species of scale-bearing nanoflagellates from four classes and 17 genera were illustrated, namely: Chrysophyceae - *Apedinella*, *Chrysolepidomonas*, *Meringosphaera*, *Paraphysomonas* (8 spp); Prymnesiophyceae - *Chrysochromulina* (32 spp), *Corymbellus*, *Imantonia*, *Pavlova*, *Phaeocystis* (2 spp), *Prymnesium* (2 spp); Prasinophyceae - *Dolichomastix* (2 spp), *Mamiella*, *Mantomiella*, *Pyramimonas* (7 spp); Dinophyceae - *Heterocapsa*; and, of uncertain taxonomic affinities, *Petasaria* and *Thaumatomastix* (3 spp).

Seventeen of the nanoflagellate species found were new records for Australian waters, specifically: *Chrysochromulina acantha, C. ahrengotii, C.* aff. *brachycylindra, C.* aff. *camella, C. mactra, C.* aff. *scutellum, Pavlova pinguis, Chrysolepidomonas* cf. *marina, Paraphysomonas antarctica, P. bandaiensis, P. foraminfera, P.* cf. *takahashii, Dolichomastix nummulifera, D.* aff. *tenuilepis, Mamiella gilva, Petasaria heterolepsis* and *Thaumatomastix* cf. *thomseni.* All species, except one (*Chrysochromulina novae-zelandiae*), are also known from the northern hemisphere.

Two known toxic species, *Chysochromulina polylepsis* and *C. leadbeateri,* responsible for massive fish kills in Scandinavia, were found in this survey, as well as the known fish-killing species, *Prymnesium patelliferum*, observed from two important Tasmanian oyster-growing area, and subsequently cultured.

The biodiversity of scale-bearing nanoflagellates in Tasmanian waters was highlighted by the large number of previously unreported scale types seen, including over 17 *Chrysochromulina*-like scale types, five prasinophyte box scale types and five *Thaumatomastix*-like scale types, but lack of complete cells prevented new species decriptions. However, sufficient material was available to fully characterise two new *Chrysochromulina* species. Full species descriptions will be prepared in the primary literature in the near future.

Over 20 unialgal strains, with representatives from each observed class, were isolated from enrichment cultures and maintained; these included *Chrysochromulina* (8 strains), *Pavlova* (4 strains), *Prymnesium* (6 strains), *Phaeocystis globosa*, *Pyramimonas grossii*, *Heterocapsa rotundata* and *Chrysolepidomonas* cf. *marina*.

Toxicity testing of 26 scale-bearing nanoflagellate strains was undertaken, using larval brine shrimp (*Artemia*) bioassays. *Prymnesium patelliferum*, was found to be toxic, with 100% mortality of *Artemia* nauplii after 24 hours exposure to stationary phase cultures, and toxicity of this species was enhanced when grown in phosphate-deplete media. *Heterocapsa rotundata* caused low percentage mortality (12.5%) of *Artemia* nauplii under the same bioassay conditions. This is a novel record of toxicity for this widespread dinoflagellate, and agrees with recent confirmation of toxicity to bivalves by the related species, *H. circularisquama*. None of the other nanoflagellate strains tested were found to be toxic.

A potential new live food species for aquaculture, *Pavlova pinguis*, was identified using a combination of ultrastructural and morphological features. This species is now used in commercial Tasmanian oyster hatcheries as a valuable algal diet.

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1. INTRODUCTION

Nanoflagellates are the "hidden" flora of phytoplankton populations. These small organisms, 2 - 20 μm in size, are largely unobserved when standard methods are used for plankton collection, preservation and examination under the light microscope. However, they are a major component of the phytoplankton, accounting for up to 90% of the total biomass and responsible for more than 50% of primary productivity (Malone, 1980; Jeffrey and Hallegraeff, 1990).

Improved methods of phytoplankton sampling and fixation, combined with extensive use of the electron microscope, have resulted in the recognition and description of many nanoflagellates, as well as increased knowledge of their distribution. Studies from tropical, temperate, subpolar and polar waters, in both northern and southern hemispheres, have shown that nanoflagellates occur in oceanic and coastal environments world-wide (Moestrup, 1979; Booth et al, 1982; Buck and Garrison, 1983; Hallegraeff, 1983; Estep et al, 1984; Hoepffner and Haas, 1990; Smith and Hobson, 1994). The majority of these studies have been from temperate waters in the northern hemisphere (Table 1.1).

Nanoflagellates are an important food source for zooplankton, including larval stages of molluscs, crustaceans and finfish, both in nature and in aquaculture operations. However, nanoflagellate blooms can also cause invertebrate and fish mortalities due to the formation of anoxic conditions or the production of toxins.

1.1 Biodiversity of Scale-bearing Nanoflagellates

The present study focuses on those marine nanoflagellates which have minute scales covering the cell, and in some cases, the flagella. Scale-bearing nanoflagellates are represented in several algal divisions, including the Chrysophyta, Haptophyta, Chlorophyta (Prasinophyceae), Dinophyta and Cryptophyta. There is an enormous variety of scale size and structure, ranging from long spined scales, over 30 μm in length, to tiny delicate flagellar scales, less than 0.3 μm. Certain scale types are unique to particular species or genera, and consequently, scales are important taxonomic characters.

In the Chrysophyceae, silica scales are found in at least two nanoflagellate genera, *Paraphysomonas* and *Polylepidomonas*, while organic scales are found in *Apedinella*, *Parapedinella* and *Chrysolepidomonas*. Organic or calcified scales (coccoliths) are commonly found in the Prymnesiophyceae, and a single cell may have up to four different types of scales. The presence of scales in the Prasinophyceae is a distinguishing feature of the class, with different types of organic scales found on both the cell body and the flagella, usually in multiple layers. Some cryptomonad species have small rosette-shaped organic scales on their flagella (Pennick, 1981; Kugrens and Lee, 1986). Even a few dinoflagellates, for example, members of the genus *Heterocapsa*, have small, species-specific body scales (Morrill and Loeblich, 1983).

In Australia, very little is known about scale-bearing nanoflagellates in the marine environment. Comprehensive studies by Beech (1983), Hallegraeff (1983) and McFadden et al (1986) provided an introduction to the diversity of scale-bearing nanoflagellates found in Victorian coastal waters and the East Australian Current, and identified over 30 prymnesiophyte, 16 prasinophyte and 5 chrysophyte species.

In the present study, a survey of scale-bearing nanoflagellates from southern Tasmanian coastal waters was undertaken, using transmission electron microscopy to examine scale morphology and thus identify species. Results were compared with literature records, both from mainland Australia and overseas.

Table 1.1: Nanoflagellate surveys from the northern and southern hemispheres.

AREA SURVEYED	REFERENCE
Northern hemisphere	
Gulf of Alaska	Booth et al, 1982
Northern Baltic Sea	Hadju et al, 1996
Western Baltic Sea	Jochem, 1990
Norwegian coastal waters	Throndsen, 1969; Leadbeater, 1972
Danish coastal waters	Manton and Leadbeater, 1974
Canada (western fjord)	Smith and Hobson, 1994
Adriatic Sea	Leadbeater, 1974
Bay of Algiers, Mediterranean Sea	Leadbeater, 1974
Gulf of Aqaba, Red Sea	Thomsen, 1978
North Atlantic Ocean	Estep et al, 1984
Central North Pacific Ocean	Hoepffner and Haas, 1990
Southern hemisphere	
East Australian Current	Hallegraeff, 1983
South-east Australian coastal waters	Beech, 1983; McFadden et al, 1986
New Zealand coastal waters	Moestrup, 1979; Rhodes et al, 1996
Weddell Sea	Buck and Garrison, 1983
Antarctic marine waters	Marchant, 1993

1.2 Culture of Scale-bearing Nanoflagellates

Due to their small size and fragility, scale-bearing nanoflagellates are often difficult to isolate and maintain in unialgal culture. While some species respond to standard enrichment media and will easily grow under laboratory conditions, other species have specific nutrient and environmental requirements. Mixotrophy is widespread in prymnesiophytes, particularly the genus *Chrysochromulina* (Jones et al, 1994), while colourless nanoflagellates, such as *Paraphysomonas* and *Thaumatomastix* species, are heterotrophic (Beech and Moestrup, 1986; Preisig et al, 1991). Of the 55 described species of *Chrysochromulina*, less than half have been cultured.

The ability to culture nanoflagellates allows an insight into their environmental growth conditions. It also means that there is a continuous supply of material for further investigations; for example, biochemical and physiological studies, taxonomic comparisons, life cycle research, toxicity testing, and evaluation of potential use for aquaculture or biotechnology (Blackburn et al, 1997).

In the present study, enrichment cultures, using standard and modified culture media, were routinely established from collected water samples. Individual cells were usually isolated by micro-pipetting, and successful unialgal cultures were maintained in the CSIRO Living Collection of Microalgae.

1.3 Toxicity of Scale-bearing Nanoflagellates

Harmful algal blooms of the prymnesiophytes, *Chrysochromulina* and *Prymnesium*, have caused massive fish mortalities, mostly in temperate waters, resulting in substantial economic losses (Edvardsen and Paasche, 1998). The 1988 *Chrysochromulina polylepis* bloom in the Kattegat-Skagerrak region, Scandinavia, affected over 60,000 km^2 of open water with cell concentrations reaching 2×10^5 cells mL^{-1}, and had a tremendous impact on marine ecosystems. Over 900 tonnes of farmed salmonid fish were killed during the bloom, which also caused severe damage to endemic marine flora and fauna (Dahl et al, 1989). A bloom of *C. leadbeateri* in northern Norway in 1991 affected mainly farmed fish, and in 1992, a bloom consisting of several *Chrysochromulina* species in Danish and German waters also resulted in fish mortalities (Aune et al, 1992; Hansen et al, 1995). In Canada, farmed rainbow trout were killed during a 1996 bloom of *C. birgeri* (Edvardsen and Paasche, 1998).

While harmful algal blooms of *Chrysochromulina* seem to be exceptional events, occurring only in coastal waters adjacent to the North Atlantic Ocean (Denmark, Finland, Norway, Sweden and Canada), *Prymnesium* blooms are recurrent in many parts of the world, usually in brackish water localities, and are responsible for numerous fish kills (Edvardsen and Paasche, 1998). *Prymnesium parvum*, *P. patelliferum* and *P. saltans* are the known causative organisms, although all *Prymnesium* species are suspected to be toxic. A bloom of *P. calathiferum* in New Zealand in 1983, resulting in fish and shellfish mortalities, was the first instance of a *Prymnesium* bloom in a fully marine environment (Chang, 1985).

In addition, blooms of "foam-producing" *Phaeocystis* have caused extensive problems for fishing industries and tourism, and may also produce toxins responsible for fish kills (Moestrup and Thomsen, 1995). Recent blooms of the scale-bearing dinoflagellate, *Heterocapsa circularisquama*, in Japan have had lethal effects on cultured bivalves, notably pearl oysters (Horiguchi, 1995; Honjo et al, 1998).

In Australia, the only reported harmful blooms of scale-bearing nanoflagellates have been those of *Prymnesium parvum* related to recurrent fish kills in the Vasse-Wonnerup estuary, Western Australia (Hallegraeff, 1992). In the present study, emphasis was placed on identifying potentially toxic species from Tasmanian coastal waters.

1.4 Scale-bearing Nanoflagellates Used as Live Feeds

At least ten species of scale-bearing nanoflagellates are routinely used world-wide as live feeds in aquaculture, including *Isochrysis* spp., *Pavlova lutheri*, *P. salina*, *Tetraselmis suecica*, *T. chuii*, and *Rhodomonas salina* (Coutteau, 1996). These are either fed directly to larval molluscs, crustacea or fish, or to brine shrimp (*Artemia*), rotifers (*Brachionus*) or copepods, which in turn are fed to crustacean and fish larvae (Brown et al, 1989). The success of a species as a live feed depends on its cell size and digestability, as well as its nutritional quality and its tolerance to temperature, light and salinity, especially if grown in outdoor tanks or ponds (Jeffrey et al, 1990; 1992).

The prymnesiophytes, *Pavlova lutheri* and *Isochrysis* sp., are frequently used in Australian hatcheries (Jeffrey et al, 1994). They have excellent nutritional profiles for larval animals, and can be cultured in mass quantities (Brown et al, 1993). However, *Pavlova lutheri* is more suited to temperate conditions, while *Isochrysis* sp. grows better at warmer temperatures (Jeffrey et al, 1992). Both strains are originally

from the northern hemisphere. Given the wide range of climatic zones for Australian aquaculture (with sea surface temperatures ranging from over 30°C in northern Australia to less than 10°C in southern waters), there is a need to find new Australian strains with equivalent nutritional value, but better adapted for local environmental conditions. In Tasmania, industry demand is for cold-water strains, able to grow in outdoor pond cultures and suitable for feeding to Pacific oyster larvae and spat (Jeffrey et al, 1994). As part of the present study, nanoflagellates which grew at 15°C or below, and had potential as live feed species, were identified.

1.5 Function of Nanoflagellate Scales

The function of scales is largely unknown. It has been suggested that they may have a protective role by isolating the cell membrane from the immediate environment, thus shielding the cell from mechanical, chemical or osmotic shock (Young, 1994).

Estep and McIntyre (1989) proposed that the scaly covering of prymnesiophytes may protect cells against toxins released into the environment by other planktonic cells. Protection from parasites (fungi, protozoa, and bacteria) and viruses has been suggested as function of prasinophyte scales (Becker et al, 1994). Long spine scales, found in some prymnesiophyte and chrysophyte species, increase the overall cell size, and may prevent predation by smaller zooplankton (Young, 1994).

In contrast, the long spine scales of the mixotrophic prymnesiophyte, *Chrysochromulina spinifera*, act as a food-collecting device, whereby particles adhering to the spines are collected periodically by the haptonema, then transported to the posterior end of the cell where they are ingested (Inouye and Kawachi, 1994).

Other possible functions of scales include providing structural support, regulating the position of the cell in the water column, and shielding the chloroplasts from high light intensities (Estep et al, 1984; Young, 1994). In the present study, scales were seen as important taxonomic characters used to identify nanoflagellate taxa from southern Tasmanian waters.

1.6 Objectives

- To undertake a taxonomic survey of southern Tasmanian coastal waters for scale-bearing nanoflagellates from the Chrysophyta, Haptophyta (exclusive of coccolithophorids), Chlorophyta (Prasinophyceae), and Dinophyta.

- To identify nanoflagellate species using transmission electron microscopy to examine scale morphology (and ultrastructure, where appropriate), and to compare scale morphology of nanoflagellates found in this survey with literature descriptions.

- To add to the current knowledge of biodiversity of nanoflagellate flora from Australian waters, and to compare nanoflagellate distribution with literature records.

- To establish enrichment cultures, and where possible, to isolate and maintain strains in unialgal culture.

- To nominate species as possible live feeds for Pacific oyster larvae and spat, based on growth in culture at 15°C or less, non-toxicity, and potential nutritional value.

- To test for toxicity of cultured strains using larval brine shrimp (*Artemia*) bioassays, and to identify potentially toxic species, blooms of which may be detrimental to the Tasmanian aquaculture industry.

2. METHODS

2.1 Sample Locations

Fifty-two water samples were collected from 21 coastal sites in southern Tasmania between June 1994 and June 1995 (Fig. 2.1). Sampling locations and dates are recorded in Table 2.1.

Samples were taken from or near aquaculture leases, as well as from river estuaries (Derwent River, Crayfish Point, Port Huon), open bays and beaches (Roches Beach, Eaglehawk Neck, South Cape Bay, Ocean Beach) and sheltered bays (Coles Bay, Honey Moon Bay, Spring Beach, Southport). Sampling was mostly shore-based, with seven samples collected from off-shore waters (Storm Bay, Pirates Bay and Maria Island).

2.2 Sample Preparation and Examination

Water samples (4 - 5 L) were generally collected from the surface. Pipeclay Lagoon and Little Swanport samples were taken from water pumped to oyster nurseries. Water temperature at the time of sampling was recorded, when possible, for each site (Table 2.1).

Water samples were treated as soon as possible after collection, usually within 1 - 3 hours (Fig. 2.2). They were first screened through a 20 μm mesh net to remove larger particles, and then concentrated using a continuous plankton centrifuge (Throndsen, 1978) at a flow rate of 100 mL min^{-1}. Approximately 2 L of water was centrifuged, resulting in an 8 - 10 mL sample concentrate. The remaining 2 - 3 L of water was used for preparation of enrichment media.

The concentrated sample was allowed to stand for 30 - 60 minutes in a 10 mL glass centrifuge tube, before examining briefly under the light microscope (x40 and x200) for motile cells. A drop taken from the meniscus was usually compared with a drop taken from the water column, and an appropriate drop was placed onto formvar-coated TEM grids (100 hex.) using a micro-pipette. Samples were fixed with 2% OsO_4 vapour (15 - 25 s) and allowed to settle for about 10 - 15 mins before excess liquid was removed using dental absorbant points. Grids were then dried at about 40°C for 10 mins, washed three times in distilled water, and allowed to air dry.

Grids were shadow cast with Au:Pd (60:40) at an approximate 30° angle in a Dynavac coating unit, or negatively stained with 2% uranyl acetate (3 mins staining followed by washing in double distilled water and air drying). Both techniques enhance the detail of surface features, with uranyl acetate staining used for particularly fine structural detail.

Initial problems with the formvar coating on TEM grids continually tearing due to continual handling were overcome by attaching grid rims to a strip of sticky tape attached to a slide edge (Moestrup and Thomsen, 1980). Problems with removing excess liquid from grids were solved by using dental absorbant points, found to be preferable to filter paper strips as they absorbed liquid more slowly, with less disturbance of cells.

Grids were examined in a Philips 410 transmission electron microscope at 60 kV for whole cells as well as nanoflagellate scales.

Fig. 2.1: Sampling locations in southern Tasmania

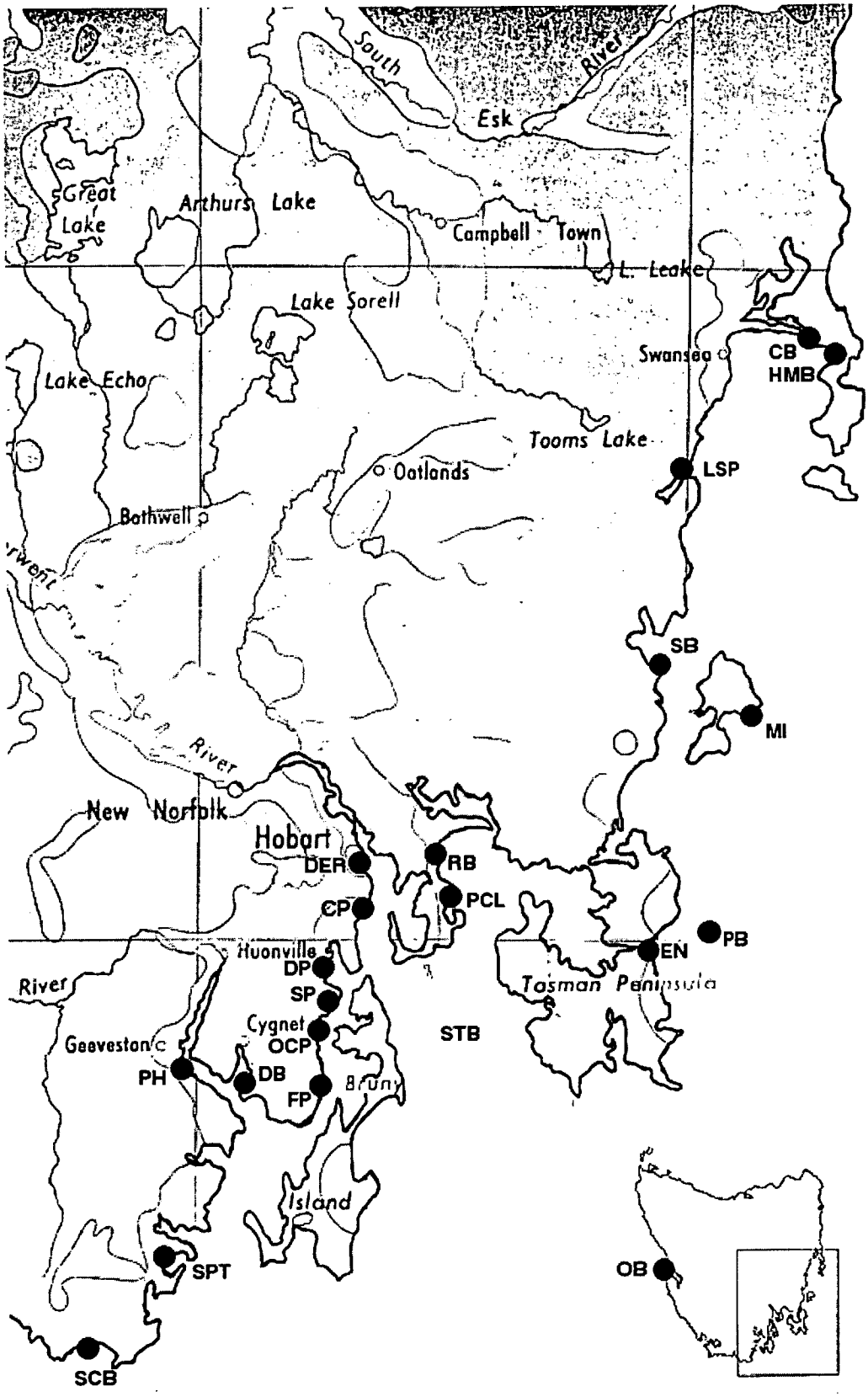
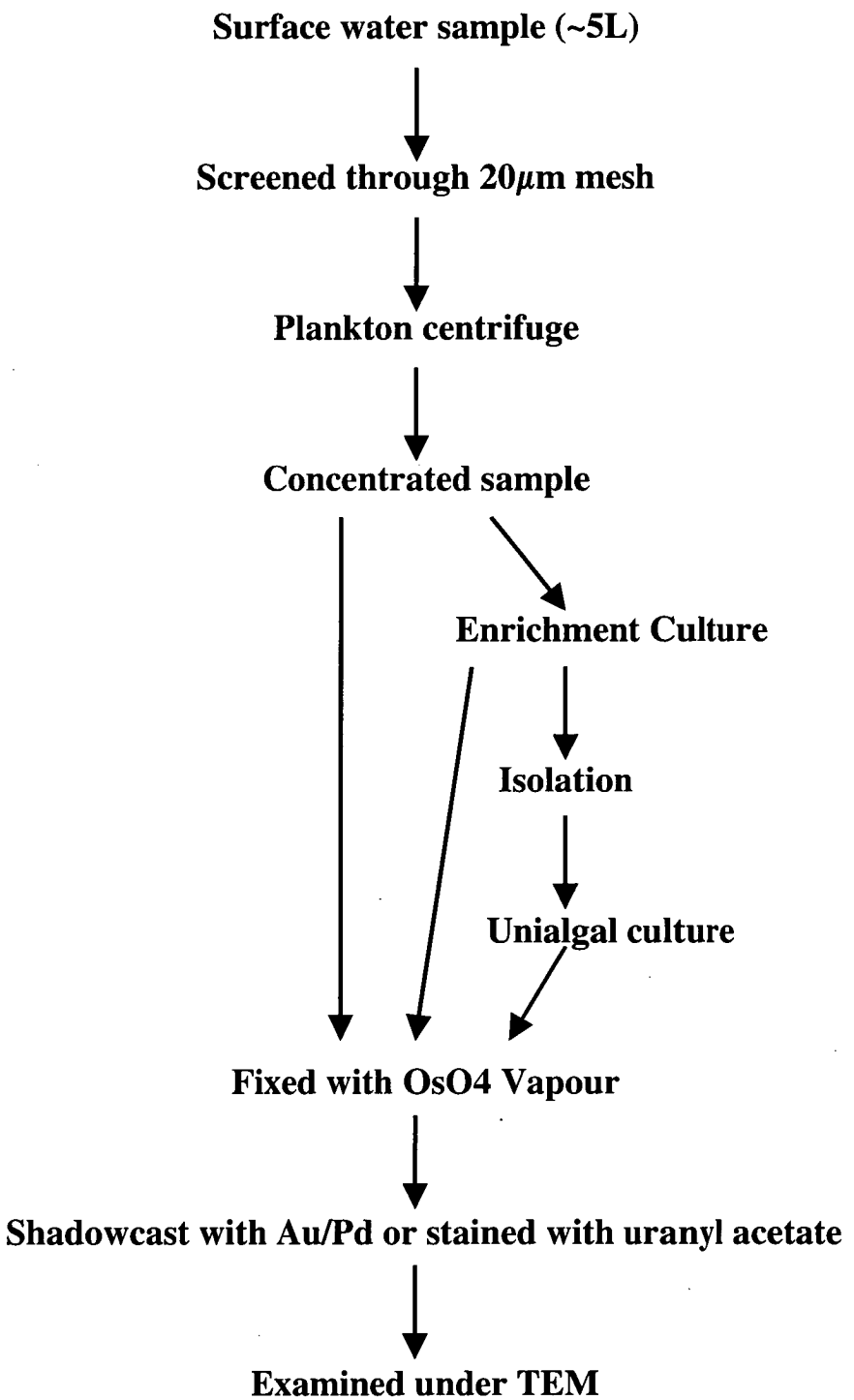


Table 2.1: Sampling location, date and water temperature.

AREA/SITE	SITE CODE	NO. OF SAMPLES	DATE SAMPLED	WATER TEMP. (°C)
Derwent River and Estuary				
Derwent River (CSIRO wharf)	DER	18	1/6/94 - 30/5/95	10.5 - 18.3
Crayfish Point	CP	1	10/4/95	ND
Storm Bay [1]	STB	3	6/5/95 - 8/5/95	ND
D'Entrecastreaux Channel				
Dru Point [2]	DP	3	1/9/94 - 19/1/95	10.5 - 20.0
Simmons Point [2]	SP	1	27/3/95	16.2
Oyster Cove Point [2]	OCP	1	9/10/94	13.0
Fleurty Point [2]	FP	1	9/10/94	12.5
Huon Estuary				
Deep Bay [2]	DB	1	9/10/94	13.0
Port Huon	PH	1	8/2/95	15.5
Southwest Coast				
Southport	SPT	1	8/3/95	19.8
South Cape Bay	SCB	1	20/2/95	ND
South Arm				
Pipeclay Lagoon [2]	PCL	8	2/6/94 - 16/3/95	9.0 - 20.0
Roches Beach	RB	1	18/10/94	18.5
Tasman Peninsula				
Eaglehawk Neck	EN	1	10/3/95	ND
Pirates Bay [1]	PB	3	26/12/94	ND
East Coast				
Spring Beach	SB	1	9/6/95	13.0
Maria Island [1]	MI	1	10/5/95	ND
Little Swanport [2]	LSP	1	26/1/95	19.0
Coles Bay	CB	1	8/3/95	ND
Honey Moon Bay	HMB	2	25/2/95; 18/4/95	20.0; 14.8
West Coast				
Ocean Beach	OB	1	4/2/95	ND

ND = Not determined [1] Off-shore samples [2] Samples taken from or near aquaculture leases

Fig. 2.2: Flowchart of water sample treatment and preparation for TEM



2.3 Enrichment Cultures

One mL of the concentrated sample, obtained after continuous centrifuging, was inoculated into enrichment media, including f, GSe, K, modified L (ML), and various dilutions of these. For example, GSe/2 was at half the nutrient concentration of the original media, and f/10 was at one tenth of the original nutrient concentration. Media compositions are given in Table 2.2, and were slightly modified from the original media recipes (Guillard, 1975; Keller et al, 1987; Bolch et al, 1991). Another modified medium used was f - Si, which was f medium lacking additional silicate.

Modified L medium was substantially different from the original L1 enrichment medium (Guillard and Hargraves, 1993) in that it had lower concentrations of major nutrients, approximately one tenth the concentration of trace elements and vitamins, contained glycerophosphate instead of inorganic phosphate, and lacked the trace elements, K_2CrO_4 and Na_3VO_4 (Table 2.3).

Germanium dioxide (GeO_2) was often added to enrichment media at low concentrations, 4×10^{-4} mg mL^{-1}, to prevent diatom growth by inhibiting silica uptake required for diatom cell wall formation (Lewin, 1966; Markham and Hagmeier, 1982).

Media preparation details are given in Jeffrey and LeRoi, 1997.

2.3.1 Water quality

Enrichment media were usually prepared using full-strength salinity seawater, except for GSe medium which consisted of 75% seawater and 25% distilled water. Water from the original collection site was used as the basis for most enrichment media, after screening through 20 μm mesh and filtering through a 0.45 μm Millipore™ filter. Small quantities were filtered sterilised (0.22 μm); larger quantities were autoclaved in teflon containers. Where seawater was not collected from the original site, oceanic seawater from Maria Island, treated with charcoal, filtered (0.45 μm) and autoclaved in teflon, was used as the basis for enrichment media. Teflon is an inert material and was less likely than glass to leach contaminating substances, which may possibly inhibit algal growth, into the media (Keller et al, 1987).

2.3.2 Growth temperature

Growth temperatures were either 10°C, 15°C, 18°C or 20 °C, depending on water temperature of the original sample.

2.3.3 Light intensity and photoperiod

Water samples were usually collected from the surface which receives maximum sunlight. Selected enrichment cultures were incubated at light intensities of 40 - 80 μmol photons PAR $m^{-2}s^{-1}$, measured with a QSL-100 light meter (Biospherical Instruments). Cool white or Daylight fluorescent tubes (20W) were used as the light source. Photoperiod consisted of 12:12 h light:dark cycles.

2.3.4 Culture vessels

Enrichment cultures were grown in 50 mm petri dishes containing c. 10 mL media. Cultures were readily checked for growth using a Nikon inverted light microscope at magnifications of x40 - 400. Selected enrichments were made in 10 - 15 mL glass test-tubes (pre-sterilised by autoclaving with Milli-Q™ water to remove toxic substances from the glass). To check for algal growth, drops were removed aseptically from both the meniscus and the water column, placed on a slide and examined microscopically at magnifications of x100 - 400.

Enrichment cultures were checked regularly (every 2 - 5 days) and evaluated for overall health, species composition and further isolation (as required).

2.3.5 Isolations

Single-cell isolations were made by micro-pipetting cells, washing in drops of sterile media on a slide, and inoculating into small quantities of media in 35 or 50 mm petri dishes (LeRoi and Bolch, 1991). Usually isolations were made from an enrichment culture rather than a direct sample, as a single cell isolation was more likely to be successful once the required species was already growing in culture.

If a species was dominant in an enrichment culture, a serial dilution technique (LeRoi and Bolch, 1991) was sometimes used in combination with micro-pipetting to obtain a unialgal culture.

Successful unialgal isolates were maintained in the CSIRO Collection of Living Microalgae in accordance with standard culturing practices (Jeffrey and LeRoi, 1997). Most isolates required subculturing every 2 weeks.

Table 2.2: Micro-molar concentrations of nutrients used in algal enrichment media.
(All concentrations in μmol L^{-1})

	f [1]	GSe [2]	K [3]	ML
Major Nutrients				
Nitrate	1760	1970	883	35
Ammonium	-	-	50	10
Urea	-	-	-	10
Phosphate	72.4	200		-
Glycerophosphate	-	-	10	30
Silicate	107	-	54	-
Chelators				
$FeCl_3$	-	1.07	-	11.7
FeEDTA	-	-	11.7	-
Na_2EDTA	-	16.1	100	1.17
Ferric citrate/citric acid	26.9/42.8	-	-	-
Trace Elements				
$MnCl_2$	1.82	4.35	0.9	0.09
$ZnSO_4$	0.15	-	0.08	0.008
$CoCl_2$	0.093	0.11	0.05	0.005
$CuSO_4$	0.079	-	0.01	0.001
Na_2MoO_4	0.052	-	0.03	0.009
$NiSO_4$	-	-	-	0.001
H_3BO_3	-	111.0	-	-
$ZnCl_2$	-	0.44	-	-
H_2SeO_3	-	0.01	0.01	0.001
Vitamins				
Thiamine	0.6	2.96	0.3	0.03
Biotin	0.004	0.008	0.002	0.0002
B_{12}	0.0008	0.0008	0.0004	0.00004
Soil Extract	-	+	-	+
Tris Buffer	-	-	1000	-

[1] Guillard (1975) [2] Bolch et al, 1991 [3] Keller et al (1987)

Table 2.3: Comparison of micro-molar concentrations of nutrients used in L1 and modified L (ML) media.[1]
(All concentrations in μmol L^{-1})

	L1	ML
Major Nutrients		
Nitrate	875	35
Ammonium	250	10
Urea	250	10
Phosphate	36.3	-
Glycerophosphate	-	30
Silicate	107	-
Chelators		
$FeCl_3$	11.7	11.7
FeEDTA	-	-
Na_2EDTA	1.17	1.17
Ferric citrate/citric acid	-	-
Trace Elements		
$MnCl_2$	0.9	0.09
$ZnSO_4$	0.08	0.008
$CoCl_2$	0.05	0.005
$CuSO_4$	0.01	0.001
Na_2MoO_4	0.09	0.009
$NiSO_4$	0.01	0.001
H_3BO_3	-	-
$ZnCl_2$	-	-
K_2CrO_4	0.01	-
Na_3VO_4	0.01	-
H_2SeO_3	0.01	0.001
Vitamins		
Thiamine	0.3	0.03
Biotin	0.002	0.0002
B_{12}	0.0004	0.00004
Soil Extract	-	+
Tris Buffer	-	-

[1] Guillard and Hargraves, 1993

2.4 Ultrastructural Studies

Some prymnesiophyte species belonging to the order Pavlovales have minute "knob" scales found on the cell body and on the flagella (Green, 1980). Due to their size, these scales are difficult to differentiate between species, even using transmission electron microscopy, and thus other characteristics are required for species identification. To identify strains of *Pavlova pinguis* (CS-375/1 and CS-375/5), it was necessary to examine ultrastructure by looking at stained thin sections under TEM.

Cultures were grown in GSe medium at 15°C under standard conditions and were in late logarithmic phase. One semi-simultaneous and two standard fixation protocols were trialled, with the following protocol being the most successful in terms of retaining cell structure.

Two mL of culture was fixed with 2 mL of 2% OsO_4 for 1 min and subsequently centrifuged to form a pellet. The supernatant was removed and 4 mL glutaraldehyde (2.5% in 0.1 M PO_4) and sucrose (0.25 g mL^{-1} glutaraldehyde) was added. Preparations were left for 45 mins before centifuging (2500 rpm for 5 mins), followed by rinsing 3 x 10 mins in buffer (0.1 M PO_4 and sucrose). Cells were then rinsed in distilled water and dehydrated in an ethanol series, starting at 30% and increasing through 50%, 70% and 100% ethanol. The dehydration was concluded with 2 x 10 mins in 100% ethanol. Pellets were left overnight in a mixture of 1 mL 100% ethanol and 1 mL Spurrs embedding resin. Finally, the cells were left for an hour in undiluted Spurrs resin before they were polymerised at 65°C for 12 hours.

Sectioning was done on a Reichhart-Jung Ultracut E ultramicrotome.

Sections were collected onto formvar-coated slot grids and stained with 0.5% uranyl acetate (in 70% methanol) for 10 mins, rinsed x4 with warm distilled water, and then stained with lead citrate for 2 mins and rinsed again x4 with warm distilled water (as thorough rinsing was important to keep grids free of contamination by uranyl acetate and lead citrate crystals). The lead citrate was freshly made up before each staining by mixing 5 mL lead nitrate (0.625 g in 10 mL distilled water and acidified with 1 drop of concentrated nitric acid) with 5 mL sodium citrate (0.83 g in 10 mL distilled water) and adding 2 mL 1.0 M NaOH to dissolve any precipitate.

Grids were examined in the TEM at 60 kV, as previously described.

3. DIVISION CHRYSOPHYCEAE: CLASS CHRYSOPHYCEAE

The Chrysophyceae are a very large and diverse class usually found in freshwater. Relatively few species are found in the marine environment, with planktonic species mostly confined to inshore waters, especially brackish sheltered areas (Throndsen, 1997).

3.1 Taxonomic Features

Flagellated chrysophytes have two anterior flagella inserted into the cell at an oblique angle to each other. One flagellum is reduced or vestigial, often with a "flagellar swelling" at its base, and may have fine hairs. The longer flagellum bears two rows of tripartite hairs, consisting of a basal region, a microtubular shaft, and a terminal filament. During swimming the longer flagellum is directed forwards, beating in an undulating fashion, and pulling the cell forwards, while the shorter flagellum is directed backwards.

Cells may be naked, or covered by scales made of silica or cellulose or forming a chitin lorica in some species. Chloroplasts, when present, are few in number (commonly one or two per cell), golden-brown, and have thylakoids in bands of three and girdle lamella. Located towards the edge of the choroplast, and closely associated with the flagellar swelling, is a stigma or eyespot, found in most motile chrysophyte cells. Colourless chrysophyte cells possess leucoplasts rather than chloroplasts, and may or may not have a stigma.

Marine representatives of scale-bearing nanoflagellates belonging to this class are found in the genera *Apedinella*, *Chrysolepidomonas*, *Paraphysomonas* and *Meringosphaera* (Table 3.1).

3.2 Australian Findings

While freshwater chrysophytes in Australian waters have been well studied (Croome and Tyler, 1985, 1988), little is known about those in the marine environment. *Apedinella spinifera*, *Meringosphaera mediterranea* and *Paraphysomonas imperforata* were reported by Hallegraeff (1983) and Beech (1983) from the East Australian Current and Victorian coastal waters. Beech also observed *Paraphysomonas butcheri* from Hobsons Bay, Victoria.

In this survey, eleven scale-bearing nanoflagellate species from the Chrysophyceae were reported. Of these, seven were new records for Australian waters and two were undescribed species of *Paraphysomonas* (Table 3.2).

In addition, four scale-bearing nanoflagellate species, including *Petasaria heterolepsis* and *Thaumatomastix* spp., having uncertain phylogenetic position within the Chrysosphyceae, were identified, and five *Thaumatomastix*-like scale types were recorded (Table 3.2).

Table 3.1: Classification of scale-bearing nanoflagellate chrysophytes from the marine environment (Throndsen, 1997).

CLASS: CHRYSOPHYCEAE *sensu* Christensen 1962

Order: Ochromonadales Pascher 1910

Family: Chrysolepidomonadaceae Peters et Andersen 1993
- single or colonial flagellate cells with organic scales

Genus: *Chrysolepidomonas* Peters and Andersen 1993
- cells with canistrate and dendritic scales covering the cell body and flagella
(1 marine species)

Family: Paraphysomonadaceae Preisig et Hibberd 1983
- cells colourless with silicified scales

Genus: *Paraphysomonas* deSaedeleer 1929
- cells covered by one or two scale types
(15 marine species)

Order: Chrysosphaerales Bourelly 1957
- predominating nonmotile stage (zoospores uniflagellated)

Family: Aurosphaeraceae Schiller 1925
- cells with silicified scales and spines

Genus: *Meringosphaera* Lohmann 1902
- cells spherical with radiating spines
(2 marine species)

Order: Pedinellales Zimmermann, Moestrup et Hällfors 1984 [1]
- radially symmetrical cells

Family: Pedinellaceae Pascher 1910
- Six, three, or no chloroplasts

Genus: *Apedinella* Throndsen 1971
- organic scales; without trailing pseudopodium
(1 marine species)

[1] Moestrup (1991) gives evidence for including the typical members of the Pedinellales in the class Dictyochophyceae.

Table 3.2: Scale-bearing nanoflagellate chrysophyte species found in southern Tasmanian waters and their overall distribution.

Species	Present Findings (Site code)	Growth in Enrichment Media	New Record for Australia	Reported Distribution
Apedinella spinifera (Throndsen) Throndsen	DER, DP, DB, PCL, EN, LS	GSe, ML	-	Polar to subtropical, coastal
Chrysolepidomonas* cf.*marina (Pienaar) Peters et Andersen	DER	GSe, ML	Yes	Temperate, coastal (one report only)
Meringosphaera mediterranea Lohmann	DER, DP, OCP, FP, SP, PCL, RB, CB, HMB	-	-	Polar to subtropical, coastal and oceanic
Paraphysomonas antarctica Takahashi	DER	GSe/2	Yes	Polar to tropical, coastal
Paraphysomonas bandaiensis Takahashi	OB	-	Yes	Temperate, freshwater (one marine record)
Paraphysomonas butcheri Pennick et Clarke	DER, CB, SP, PCL, EN, DP, HMB, OB	GSe/10, ML	-	Temperate, freshwater and marine, coastal
Paraphysomonas foraminifera Lucas	LSP, HMB	GSe, ML	Yes	Temperate to polar, coastal
Paraphysomonas imperforata Lucas	All inshore samples (except SC, SB)	ML, GSe/2, GSe/10, f-Si/100	-	Polar to subtropical, freshwater and marine, coastal
Paraphysomonas* cf. *takahashii Cronberg et Kristiansen	DER, DP, SP	-	Yes	Temperate, freshwater (one marine record)
***Paraphysomonas* spp** (2)	DER, STB	ML	Yes	
INTER SEDIS ***Petasaria heterolepis*** Moestrup	DER, PCL, EN	-	Yes	Temperate to subtropical, coastal
Thaumatomastix salina (Birch-Andersen) Beech et Moestrup	DER, PCL, DP, OCP, FP, SPT, DB, SPT, RB, LSP	-	-	Temperate, coastal
Thaumatomastix* cf. *thomseni Tong	RB, FP	-	Yes	Temperate, coastal
Thaumatomastix tripus (Takahashi et Hara) Beech et Moestrup	DER, DP, DB, PCL, EN, LSP, SP	-	-	Temperate, coastal
***Thaumatomastix* spp.** (5)	DER, OCP, OB	-	Yes	

3.3 Species Descriptions

***Apedinella spinifera* (Throndsen) Throndsen** **Figs. 3.1 - 3.3**

Synonyms: ***Apedinella radians* (Lohmann) Campbell**

Micrographs: Throndsen, 1971; Figs. 8 - 10.
Beech, 1983; Plate 22.2 A - B.
Hallegraeff, 1983; Figs. 2a - d.

Present Findings.
Isolated plate and spine scales were common, and found in samples from the Derwent River, Dru Point, Deep Bay, Pipeclay Lagoon, Eaglehawk Neck and Little Swanport.

Whole cells were seen in Derwent River samples, and also in GSe and ML enrichment cultures established from Dru Point and Deep Bay samples.

Description.
Cells (5 - 7 µm diameter) were covered with numerous plate scales and had 5 - 6 distinctive spine scales (Fig. 3.1). The single flagellum was about twice the length of the cell, and usually became detached during sample preparation.

Plate scales were oval to circular and had two distinct sizes: 2.3 - 2.5 x 1.3 µm (n=32) and 0.9 - 1.0 x 0.6 - 0.7 µm (n=32). The smaller plate scales were approximately half the size of the larger ones. Both the large and small plate scales had a faint surface pattern of irregular interwoven fibrils, a marginal band, and a slightly raised rim (Fig. 3.3). Spine scales had a triangular base with a long tapering spine, 16 - 26 µm long (n=21) (Fig. 3.2).

A. spinifera scales from different locations had similar structure, but varied in size, particularly the spine scales (Table 3.3). These ranged from 12 - 34 µm, and scales from wild samples were usually, but not always, longer than those from cultured cells. The large plate scales were generally larger than those described for the type material (Throndsen, 1971), while the small plate scales had an overall similar size range.

Distribution.

A. spinifera is a widely distributed species and has been recorded from the Arctic, Norway, Denmark, Finland, UK, Canda, Yugoslavia, Romania, Algeria, Israel, New Zealand and south-east Australia (Leadbeater 1972, and references therein; Parke and Dixon, 1976; Moestrup, 1979, and references therein; Beech, 1983; Hallegraeff, 1983; Edler et al, 1984; Jochem, 1990; Mihnea, 1993; Smith and Hobson, 1994; Throndsen, 1997).

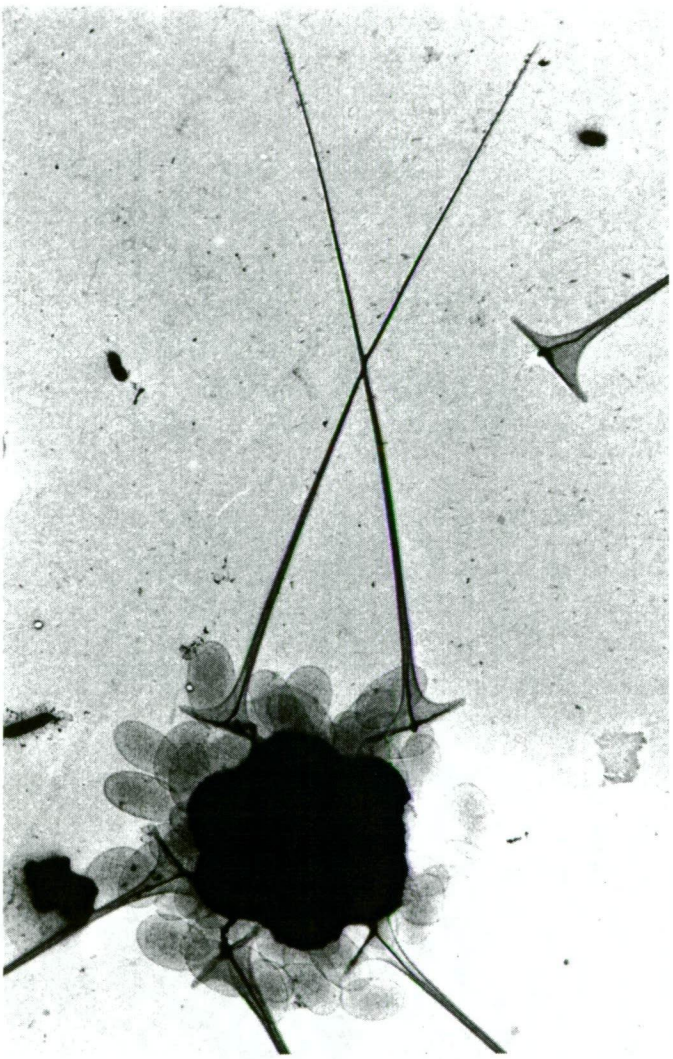


Fig. 3.1: *A. spinifera* cell (5 μm) with spine and plate scales; from a Dru Point enrichment culture

(Micrograph no. 4870)

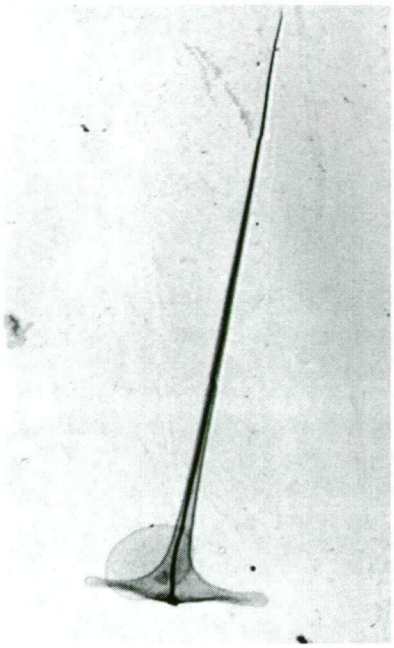


Fig. 3.2: *A. spinifera* spine scale (12 μm); from the Derwent River

(Micrograph no. 5149)

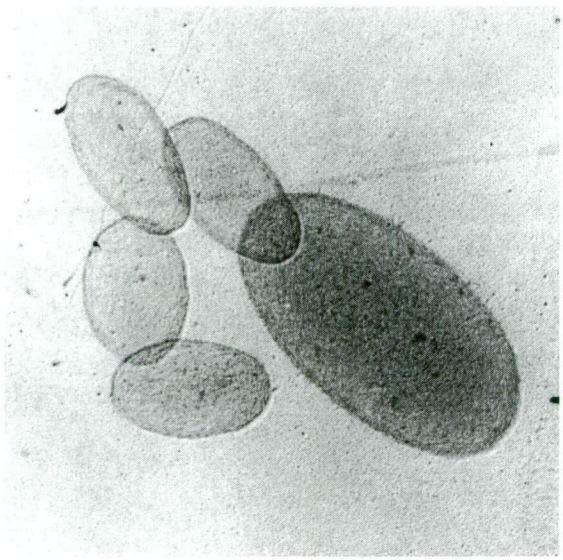


Fig. 3.3: *A. spinifera* large (2.3 x 1.3 μm) and small (1.0 x 0.6 μm) plate scales; from the Derwent River

(Micrograph no. 5147)

Table 3.3: Size of *Apedinella spinifera* scales from different locations.

SOURCE	MATERIAL EXAMINED	SPINE SCALES		LARGE PLATE SCALES	SMALL PLATE SCALES
		Length (µm)	*No./Cell*	*Dimensions (µm)*	*Dimensions (µm)*
Norway *(type)* (Throndsen, 1971)	Cultured	12 - 15 (34)	4 - 9	1.5 x 1.0	1.0 x 0.5
New Zealand (Moestrup, 1979)	Wild	22 - 32	ND	2.6 x 1.4	1.1 x 0.8
Victoria, Australia (Beech, 1983)	Wild	≤ 30	4 - 6	1.4 - 1.7 x 0.9 - 1.1 ($\bar{x}$ =1.6 x 1.0; n=3)	0.85 x 0.7 (n=1)
New South Wales, Australia (Hallegraeff, 1983)	Wild	27 - 29	4 - 6	2.5 x 1.6	1.4 x 0.8
Tasmania, Australia	Cultured	16 - 22 ($\bar{x}$ =19; n=14)	5 - 6	2.5 x 1.3 (n=24)	0.9 x 0.7 (n=24)
	Wild	12 - 26 ($\bar{x}$ =16; n=7)	ND	2.3 x 1.3 (n=8)	1.0 x 0.6 (n=8)

ND = Not determined

***Chrysolepidomonas* cf. *marina* (Pienaar) Peters et Andersen** **Figs. 3.4 - 3.6**

Synonym: ***Sphaleromantis marina* Pienaar**

Micrographs: Pienaar, 1976; Figs. 3 - 17.

Present Findings.

This species was found in Ge and ML enrichment cultures derived from two different Derwent River samples. A unialgal culture (CS-490) is currently maintained in the CSIRO Living Collection of Microalgae, in GSe medium at 15°C under standard growth conditions.

This is a new record for Australian waters.

Description.

Cells were approximately 5 μm. They had a long flagellum, which was about the length of the cell, and a short flagellum, half the cell length. Unfortunately, flagella became detached during sample preparation, and complete cells were not observed under the transmission electron microscope.

On the cell surface, two types of scales were seen (Fig. 3.4). These organic dendritic scales are characteristic of the genus (Peters and Andersen, 1993). One scale type, 0.32 - 0.35 μm in length (n=2), had eight "finger-like" projections at its distal end, and a ring of shorter projections near its base (Fig. 3.6). This scale was similar in both size and structure to the "tree-like" scales described for *Chrysolepidomonas marina* (Pienaar, 1976).

The second scale type was longer, approximately 0.6 μm, and had five shorter "finger-like" projections at its distal end. Two rings of shorter projections were present; one near the scale base, and the other about halfway between the base and the end (Fig. 3.5). These scales were not recorded for *C. marina*.

C. marina has three scale types: the "tree-like" scales previously mentioned, which are found on both the cell and flagellar surfaces; small "flower-pot" scales (0.08 μm) with five spines, found only on the cell surface; and similar canistrate scales (0.1 μm), but with four spines and found only on the flagellar surface (Pienaar, 1976). These latter two scale types were not present in the Tasmanian material.

Further examination of this material is required to determine if it is a subspecies of *C. marina* or a completely new marine species of *Chrysolepidomonas.*

Distribution.

C. marina was orginally described in enrichment cultures established from lagoon samples from San Juan Island, Washington state, USA (Pienaar, 1976). It is the only marine species in the genus and is rarely reported.

Fig. 3.4: *Chrysolepidomonas* scales on cell surface; from a Derwent River enrichment culture

(Micrograph no: 5284)

Fig. 3.5: *Chrysolepidomonas* long scale (c. 0.6 μm) with five "finger-like" projections at distal end, and two sets of shorter projections along the scale length; from a Derwent River enrichment culture

(Micrograph no: 5275)

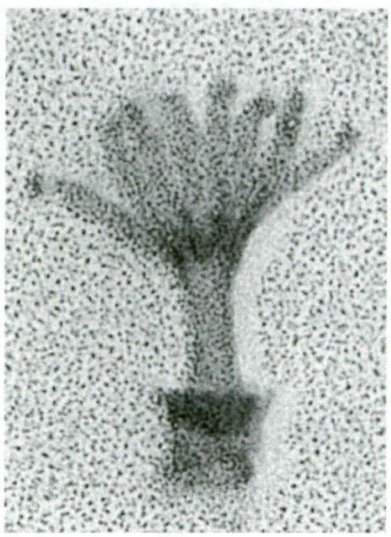


Fig. 3.6: *Chrysolepidomonas* short scale (0.35 μm) with eight "finger-like projections at distal end, and one set of shorter projections near the base; from a Derwent River enrichment culture

(Micrograph no: 5277)

***Meringosphaera mediterranea* Lohmann** **Figs. 3.7 - 3.8**

Micrographs: Leadbeater, 1974; Plate 4.
Moestrup, 1979; Figs. 2 and 6.
Booth et al, 1982; Figs. 1 - 2.
Beech, 1983; Plate 2.22 C - D.
Hallegraeff, 1983; Figs. 4a - b.
Estep et al, 1984; Fig. 34.
Hoepffner and Haas, 1990; Fig. 1.

Present Findings.
Plate and spine scales were common and were found in samples from the following sites: Derwent River, Dru Point, Oyster Cove Point, Fleurty Point, Southport, Pipeclay Lagoon, Roches Beach, Coles Bay and Honey Moon Bay.

Whole cells were seen in Oyster Cove Point and Southport samples.

Description.
Cells (approximately 6 µm diameter) were covered by elliptical plate scales and had several long distinctive spine scales.

Plate scales, 2.5 - 3.1 x 1.7 - 1.9 µm ($\bar{x}$=2.8 x 1.8 µm; n=2), were patternless and had a narrow raised central thickening (Fig. 3.8). Spine scales ranged from 14 - 19 µm in length, ($\bar{x}$=17; n=3), with a wide conical base and an undulating tapering spine (Fig. 3.7). Each spine carried 11 - 14 irregularly-spaced short barbs with points directed towards the spine tip.

Scale size varied considerably (Table 3.4). Plate scales recorded by Leadbeater (1974) from the Mediterranean Sea, and by Beech (1983) from Victorian coastal waters, were similar in size to the Tasmanian material, but those from the East Australian Current were approximately four times smaller (Hallegraeff, 1983), and those from New Zealand were half the size (Moestrup, 1979). Spine length also differed with geographical location, and it was interesting to note that the longest spine scales (30 - 40 µm) came from tropical waters (Hallegraeff, 1983).

Distribution.

M. mediterranea has been been found in coastal and oceanic waters of both hemispheres, including those of: Greenland, UK, Denmark, Yugoslavia, Israel, Alaska, Canada, New Zealand and Australia (Booth et al, 1982, and references therein; Smith and Hobson, 1994; Beech, 1983; Hallegraeff, 1983), as well as the Indian Ocean, the North and South Atlantic Oceans, and the North Pacific Ocean (Hoepffner and Haas, 1990, and references therein).

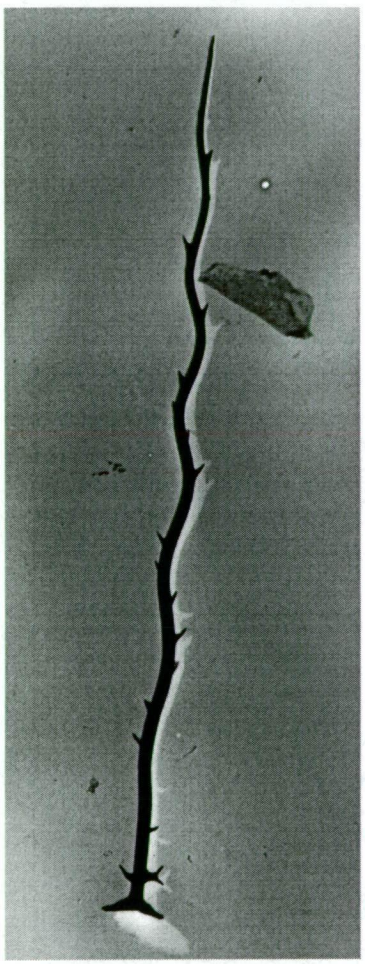


Fig. 3.7: *M. mediterranea* spine scale (17 μm); from Oyster Cove Point

(Micrograph no: 4943)

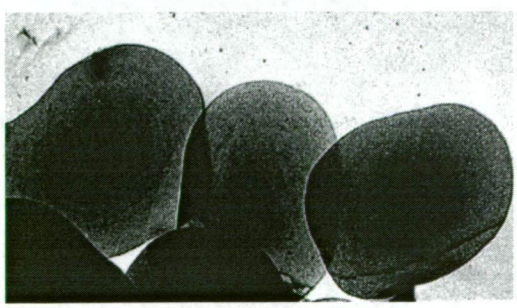


Fig. 3.8: *M. mediterranea* plate scales (2.5 - 3.1 x 1.7 - 1.9 μm); from Oyster Cove Point

(Micrograph no: 4946)

Table 3.4: *Meringosphaera mediterranea* scales from different locations.

SOURCE	SPINE SCALES		PLATE SCALES
	Length (μm)	*No. of Barbs*	*Dimensions (μm)*
Mediterranean Sea (Leadbeater, 1974)	18 - 22 (n=3)	16	2.0 - 2.5 x 1.4 - 1.6 ($\bar{x}$ =2.15 x 1.5; n=4)
New Zealand (Moestrup, 1979)	12 (n=1)	c. 12	1.8 x 1.4 (n=1)
Gulf of Alaska (Booth et al, 1982)	18 - 20 (n=4)	ND	-
North West Shelf, Australia (Hallegraeff, 1983)	30 - 40 (n=1)	c. 10	-
East Australian Current (Hallegraeff, 1983)	-	-	0.8 x 0.4 (n=1)
Victoria, Australia (Beech, 1983)	13 - 15 (n=2)	10 - 13	2.4 x 1.8 (n=1)
North Atlantic Ocean (Estep et al, 1984)	c. 15 (n=2)	ND	-
North Pacific Ocean (Hoepffner & Haas, 1990)	15 - 20	11 - 14	-
Tasmania, Australia	14 - 19 ($\bar{x}$ =17; n=3)	11 - 14	2.5 - 3.1 x 1.7 - 1.9 ($\bar{x}$ =2.8 x 1.8; n=2)

ND = Not determined

***Paraphysomonas antarctica* Takahashi** **Figs. 3.9 - 3.10**

Micrographs: Takahashi, 1987: Figs. 1 - 3.
Vørs, 1992; Fig. 30 (d) -(e).
Tong, 1997; Fig. 14 (c).

Present Findings.
Whole cells and scales were recorded from a GSe/2 enrichment culture derived from a Derwent River sample.

This is the first record of this species for Australian waters.

Description.
Cells were approximately 3 µm, with long and short flagella, 12 µm and 2 µm respectively (n=1). Flagellar hairs were seen on the long flagellum and ranged from 1.4 - 1.5 µm (Fig. 3.9).

Scales had very long spines, 1.9 - 3.1 µm in length ($\bar{x}$=2.5 µm; n=4), with a distinct thin spine tip, 0.3 - 0.45 µm ($\bar{x}$= 0.4 µm; n=10) (Fig. 3.10). The base diameter was approximately half the spine length and ranged from 1.1 - 1.4 µm ($\bar{x}$= 1.1 µm; n=4).

This description closely corresponded to previous records for this species, and scale sizes were very similar (Table 3.5).

P. antarctica scales are very similar to those of *P. imperforata*, but considerably larger in size. An abrupt change in spine width to a short thin spine tip is the main differentiating feature, and there is no central annular depression as seen in some *P. imperforata* scales.

Distribution.
P. antarctica was originally described from Antarctic ice-covered seawater (Takahashi, 1987), and has since been recorded from Denmark, the Baltic Sea, Greenland, the Caribbean Sea and the UK, at temperatures ranging from -1°C to 20°C (Vørs, 1992, and references therein; Tong, 1997).

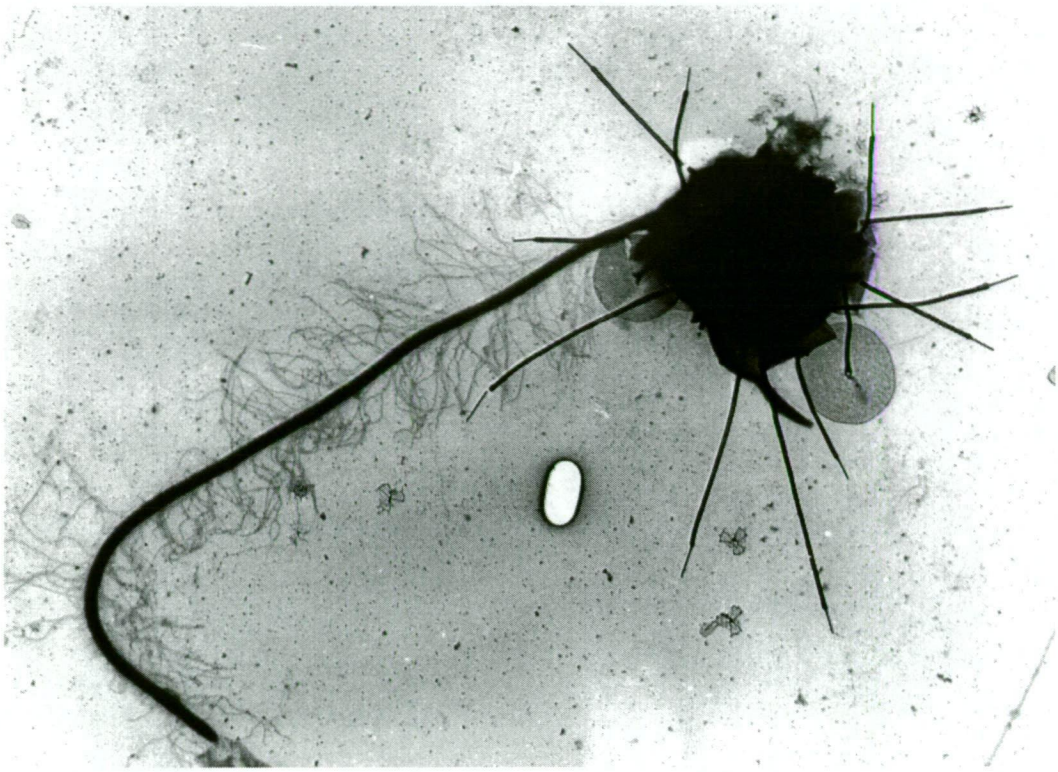


Fig. 3.9: *P. antarctica* cell (c. 3 μm); from a Derwent River enrichment culture

(Micrograph no: 4858)

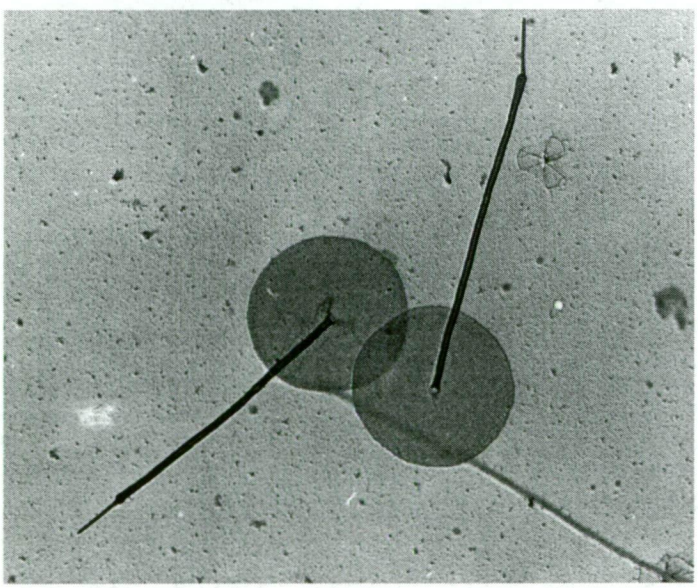


Fig. 3.10: *P. antarctica* spine scales (2.2 - 2.3 μm); from the same culture

(Micrograph no: 4860)

Table 3.5: *Paraphysomonas antarctica* from different locations.

SOURCE	CELL SIZE	FLAGELLA		FLAGELLAR	SPINE SCALES		
	(µm)	*Long (µm)*	*Short (µm)*	HAIRS	*Spine Length (µm)*	*Spine Tip (µm)*	*Base Diameter (µm)*
Antarctica *(type)* (Takahashi, 1987)	2.0 - 4.3	12.5 - 27	1.8 - 4.5	-	1.0 - 3.25	0.3 - 0.7	0.9 - 1.75
Finland (Vørs, 1992)	-	-	-	-	2.4 - 3.1	0.2 - 0.3	1.2 - 1.5
UK (Tong, 1997)	-	-	-	-	1.5 - 3.1	0.3 - 0.5	1.1 - 1.3
Tasmania, Australia	c. 3	12 (n=1)	2 (n=1)	1.4 - 1.5	1.9 - 3.1 ($\bar{x}$=2.5; n=4)	0.3 - 0.45 ($\bar{x}$ = 0.4; n=10)	1.1 - 1.4 ($\bar{x}$ = 1.1; n=4)

***Paraphysomonas bandaiensis* Takahashi** **Figs. 3.11 - 3.12**

Micrographs: Takahashi, 1976; Figs. 6 - 8.
Preisig and Hibberd, 1982a; Fig. 2G - K.
Vørs et al, 1990; Figs. 32 - 34.
Caron et al, 1999; Fig. 1A - B.

Present Findings.
Scales and a whole cell were observed in a sample from Ocean Beach on the west coast of Tasmania.

This is the first record of this species from Australian coastal waters.

Description.
The cell measured about 3 μm in diameter and had a long (10 μm) and a short (2 μm) flagellum. Flagellar hairs were not seen in this preparation. Cell dimensions and flagellar lengths were in the lower size range reported for this species (Table 3.6).

Scales had a round base plate, 0.7 - 0.8 μm in diameter (n=3), with a thickened rim. A few small perforations were arranged sporadically in a roughly concentric ring near the rim (Fig. 3.12). The central spine was 0.9 - 1.1 μm in length (n=4) and had a slight club-shaped tip (Fig. 3.11). Scale size was in the upper size range reported for this species (Table 3.6).

Two *Paraphysomonas* subspecies have scales with a thickened rim and central spine. *P. vestita* ssp. *vestita* has a thin tapering spine with an acute tip, while *P. vestita* ssp. *truncata* has distinctly truncated spine with obvious transverse striations (Preisig and Hibberd, 1982a; Fig. 1). These scales are different from those of *P. bandaiensis* with its bluntly-rounded spine tip and concentric ring of perforations around the rim. In addition, scales of *P. bandaiensis* are generally smaller, having a spine length similar to the base diameter (Table 3.6). Although scale morphology is somewhat similar, *P. bandaiensis* has been shown to be genetically distinct from *P. vestita* (Caron et al, 1999).

Distribution.
P. bandaiensis has been previously reported mostly from temperate freshwater environments (Preisig and Hibberd, 1982a, and references therein; Vørs et al, 1990), but has also been isolated from the Sargasso Sea (Caron et al, 1999).

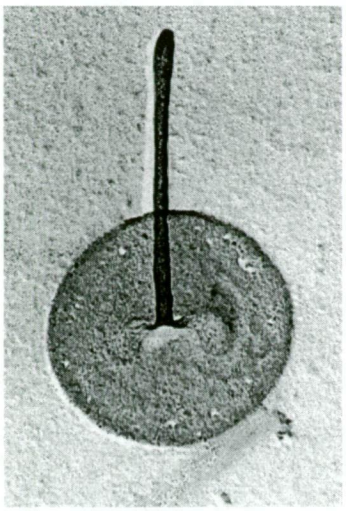


Fig. 3.11: *P. bandaiensis* spine scale (1 μm) with a club-shaped tip; from Ocean Beach

(Micrograph no: 5329)

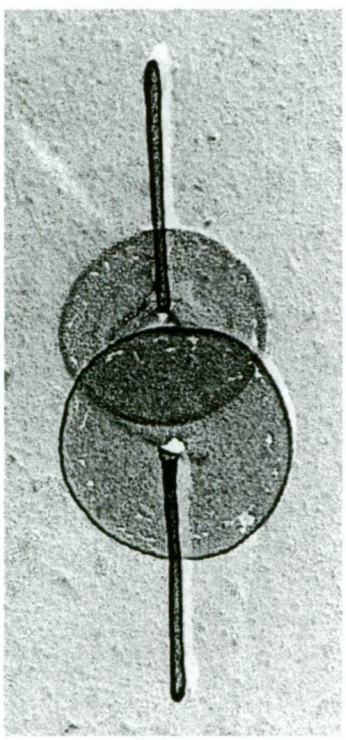


Fig. 3.12: *P. bandaiensis* spine scales (0.9 - 1.0 μm) showing outer irregular perforations from the same sample: from Ocean Beach

(Micrograph no: 5329)

Table 3.6: Comparison of *Paraphysomonas bandaiensis* and *Paraphysomonas vestita*.

SPECIES	CELL. DIAM. (μm)	FLAGELLA		SPINE SCALES		HABITAT
		Long (μm)	*Short* (μm)	*Base Diam.* (μm)	*Length* (μm)	
P. bandaiensis [1] Takahashi	4 - 8	7 - 25	2.0 - 6.5	0.2 - 0.8	0.2 - 1.1	Freshwater
P. bandaiensis (Tasmanian material)	3 (n=1)	9.8 (n=1)	1.6 (n=1)	0.7 - 0.8 ($\bar{x}$ =0.7; n=3)	0.9 - 1.1 ($\bar{x}$ =0.95; n=4)	Marine
P.vestita ssp. *truncata* [1] Preisig et Hibberd	4 - 6	9 - 15	2 - 3	0.7 - 0.9	1.0 - 1.4	Freshwater
P.vestita ssp. *vestita* [1] (Stokes) De Saedeleer	5 - 26	14 - 54	3 - 10	0.4 - 4.3	1.3 - 12.5	Freshwater Marine

[1] Preisig and Hibberd, 1982a.

***Paraphysomonas butcheri* Pennick et Clarke** **Figs. 3.13 - 3.19**

Micrographs: Pennick and Clarke, 1972; Figs. 1 - 11.
Leadbeater, 1974; Plate 5 G - H.
Thomsen, 1975; Figs. 16 - 19.
Takahashi, 1976; Figs. 14 - 16.
Moestrup, 1979; Fig. 1.
Preisig and Hibberd, 1982b; Fig. 11.
Beech, 1983; Plate 2.19 A - B.
Vørs, 1992; Fig. 30 (e).
Tong, 1997; Fig. 14 (a).
Caron et al, 1999; Fig. 1F.

Present Findings.

Whole cells and scales were commonly found in the Derwent River, and were also seen in Storm Bay, Coles Bay and Southport samples. Scales only were found in samples from Pipeclay Lagoon, Eaglehawk Neck, Dru Point and Honey Moon Bay, as well as Ocean Beach on the west coast of Tasmania.

Paraphysomonas butcheri cells grew in ML and GSe/10 enrichment cultures established from Little Swanport samples.

Description.

Cells ranged from 1.5 - 2.5 µm diameter ($\bar{x}$=2.1 µm; n=5), which was marginally less than the type species size of 2.5 - 3.0 µm (Pennick and Clarke, 1972), and also smaller than later records (Table 3.7). Each cell had a long and a short flagellum; flagellar hairs (1.0 - 1.5 µm) were clearly visible on the longer flagellum (Fig. 3.13). Flagellar lengths were slightly different to those of the type species. The longer flagellum was greater than 2.5 times the cell diameter, ranging from 7 - 10 µm ($\bar{x}$=8 µm; n=5), while the shorter flagellum was smaller than the cell diameter, ranging from 1 - 2 µm ($\bar{x}$=1.5 µm; n=6). These observations generally agreed with those made by Preisig and Hibberd (1982b) of freshwater material from the UK, as well as later observations of marine samples (Table 3.7).

Two types of scales covered the cell body: ornate crown scales and plate scales. Plate scales were usually dominant, but both types were always present on any one cell.

Crown scales consisted of a proximal ring and an arched distal ring connected by 5 - 6 evenly spaced perpendicular struts; the area enclosed by the distal ring was divided into several irregular apertures. Some scales were found with small protuberances, both on the apical ring as well as on the distal ring and the supporting struts (Fig. 3.18). These protuberances have been recorded previously, but only from marine material, and only on the apical ring (Leadbeater, 1974; Moestrup, 1979; Beech, 1983).

Crown scales had base dimensions of 0.3 - 0.4 x 0.4 - 0.65 µm ($\bar{x}$=0.6 x 0.4 µm; n=5) and a height of 0.3 µm (n=1), which agreed with previous measurements (Table 3.8).

Plate scales had a meshwork pattern of apertures forming approximate concentric rings. In contrast to the relatively consistent size and structure of the crown scales, plate scales were very variable, as shown by other records for this species (Table 3.8). At least five different forms of plate scales were seen in this study.

Elliptical scales, 0.45 - 0.7 x 0.3 - 0.6 µm ($\bar{x}$=0.6 x 0.5 µm; n=24), were the most common form. These scales generally had an outer ring of 11 - 13 large regularly arranged apertures, an inner ring of 7 - 12 apertures and a central area of 8 - 11 smaller apertures. Outer and inner concentric bands were distinct and slightly thicker, with small protuberances seen on the inner band (Fig. 3.14). These scales most closely resembled those described for the type species (Pennick and Clarke, 1972), but were slightly smaller.

The second form was also elliptical, 0.55 - 0.7 x 0.7 - 0.9 µm ($\bar{x}$=0.8 x 0.6 µm; n=10), with a distinctive outer ring of 14 - 16 large apertures and 45 - 90 smaller central perforations (Fig. 3.15). These scales were observed in Little Swanport enrichment cultures, and had some resemblance to scales described from Japan as *P. inconspicua* by Takahashi (1976; Figs. 15 - 16), but later considered as a synonym for *P. butcheri* by Preisig and Hibberd (1982b).

Another form was similar to scales described by Moestrup (1979; Fig. 1) from New Zealand. This scale type was observed from a Dru Point sample. It was elliptical, 0.6 x 0.4 µm (n=1), with nine large apertures in the outer ring, four elongate inner apertures and two small, almost circular apertures, in the central area. Outer and inner concentric bands were distinct with small protuberances seen on the inner band (Fig. 3.16).

The fourth form of plate scale was circular, 0.7 μm in diameter (n=1). It had an outer ring of 16 apertures and a central area of 33 slightly smaller apertures (Fig. 3.17), and was found in Derwent River samples.

Circular to elliptical plate scales, 0.4 - 0.7 x 0.5 - 0.8 μm ($\bar{x}$=0.7 x 0.5 μm; n=11), with c. 85 - 125 central perforations of similar size (Figs. 3.18, 3.19) were found in a Storm Bay sample, and also in the sample from Ocean Beach. This scale form matched that described by Leadbeater (1974; Plate 5G - H) from the Mediterranean Sea, and by Beech (1983; Plate 2.19A - B) from Hobson's Bay, Victoria.

Given the variation in scale morphology, genetic studies comparing different *P. butcheri* strains would be useful to show what genetic links, if any, exist within this species. *P. butcheri* has already been shown to be genetically distinct from three other morphologically similar *Paraphysomonas* species (Caron et al, 1999).

Distribution.

P. butcheri is widely distributed in temperate coastal waters and freshwater habitats and has been reported from: UK, Norway, Finland, Denmark, Netherlands, Yugoslavia, Algeria, Greece, Japan, USA and Canada in the northern hemisphere (Preisig and Hibberd, 1982b, and references therein; Smith and Hobson, 1994; Vørs, 1992, and references therein; Caron et al., 1999), as well as New Zealand, Australia and Antarctica in the southern hemisphere (Moestrup, 1979; Beech, 1983; Takahashi, 1987).

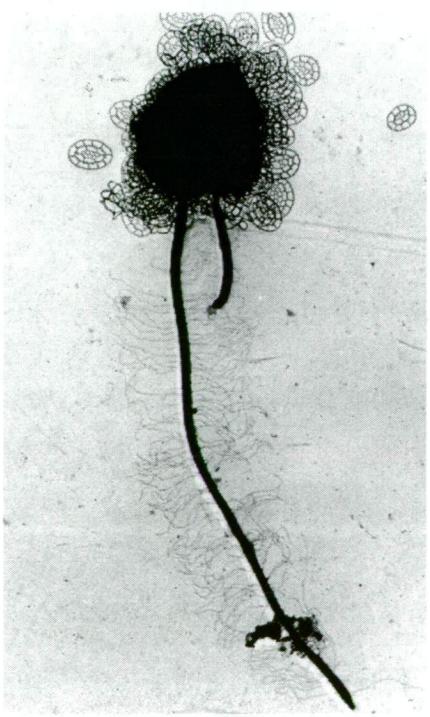


Fig. 3.13: *P. butcheri* cell (c. 2.5 μm); from the Derwent River

(Micrograph no: 5145)

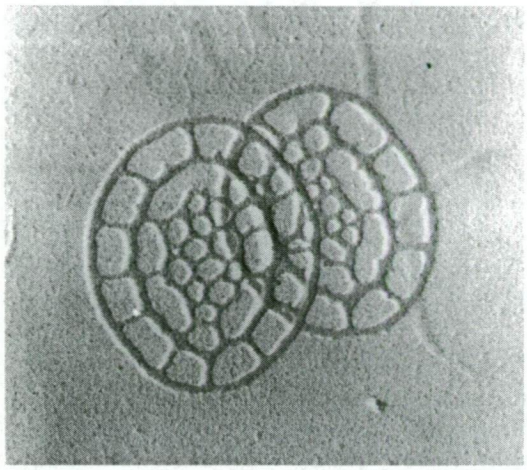


Fig. 3.14: Common *P. butcheri* plate scales (0.6 x 0.5 μm) - form 1; from the Derwent River

(Micrograph no: 4632)

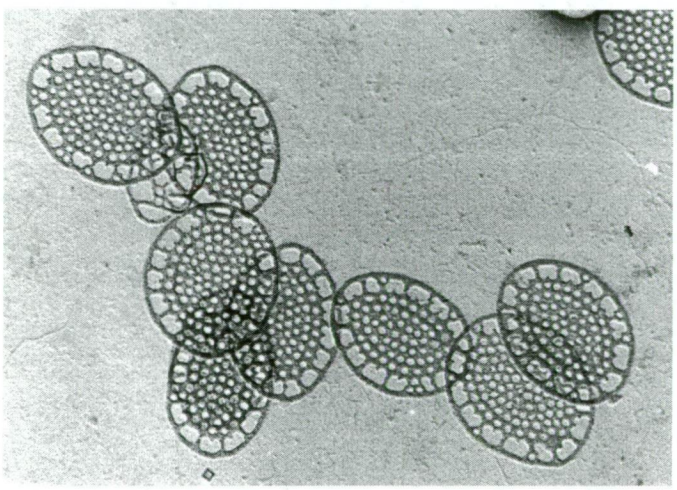


Fig. 3.15: *P. butcheri* plate scales (0.8 x 0.6 μm) - form 2; from a Little Swanport enrichment

(Micrograph no: 5306)

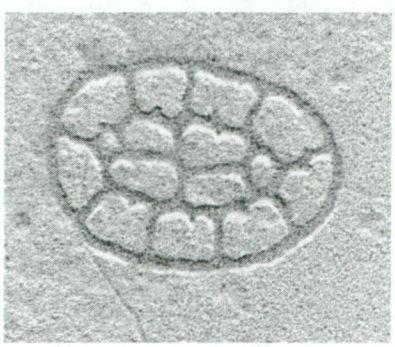


Fig. 3.16: *P. butcheri* plate scale (0.6 x 0.4 μm) - form 3; from Dru Point

(Micrograph no: 4905)

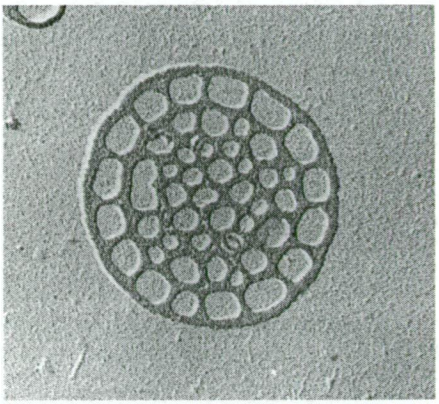


Fig. 3.17: *P. butcheri* plate scale (0.7μm) - form 4; from the Derwent River.

(Micrograph no: 4826)

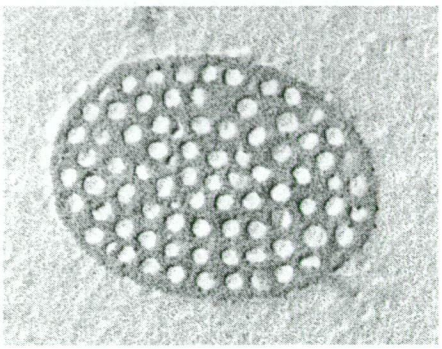


Fig. 3.19: *P. butcheri* plate scale (0.7 x 0.5 μm) - form 5; from Ocean Beach

(Micrograph no: 5328)

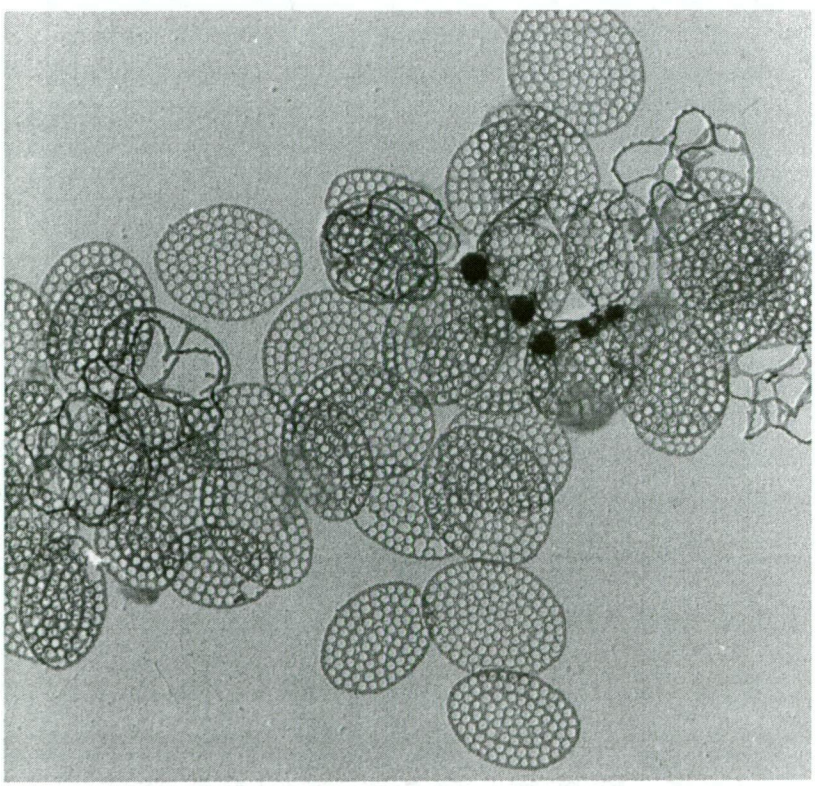


Fig. 3.18: *P. butcheri* plate scales (0.7 x 0.5 μm) - form 5; and crown scales with small protuberances on rings and struts; from Storm Bay

(Micrograph no: 5328)

Table 3.7: *Paraphysomonas butcheri* cells from different locations.

SOURCE	CELL DIMENSIONS (μm)	FLAGELLA Long (μm)	FLAGELLA Short (μm)	FLAGELLAR HAIRS (μm)	HABITAT
Essex, UK *(type)* (Pennick & Clarke 1972)	2.4 - 3.0	6 - 9	2.4 - 3.0	c. 1.4	Marine
Cambridge, UK (Preisig & Hibberd, 1982b)	3 - 4	7 - 10	2.5 - 3.0	1.4 - 1.7	Freshwater
Southhampton, UK (Tong, 1997)	3.5 - 7.5	ND	ND	ND	Marine
Finland (Vørs, 1992)	2.3	7 (n=1)	c. 2 (n=1)	-	Marine
Denmark (Thomsen, 1975)	c. 3	8.5 (n=1)	c. 2 (n=1)	1.2 - 1.4 (n=4)	Marine
Mediterranean Sea (Leadbeater, 1974)	c. 3 (n=1)	ND	c. 2 (n=1)	1.3 - 1.5 (n=3)	Marine
Victoria, Australia (Beech, 1983)	c. 2 (n=1)	c. 7 (n=1)	c. 1 (n=1)	ND	Marine
Tasmania, Australia	1.5 - 2.5 ($\bar{x}$ =2.0; n=6)	7 - 10 ($\bar{x}$ =8; n=5)	1 - 2 ($\bar{x}$ =1.5; n=9)	1.0 - 1.5 ($\bar{x}$ =1.2; n=9)	Marine

ND = Not determined

Table 3.8: *Paraphysomonas butcheri* scales from different locations.

SOURCE	DOMINANT SCALE TYPE	CROWN SCALES			PLATE SCALES	
		Base Diameter (μm)	*Height (μm)*	*Protrusions on Apical Ring*	*Dimensions (μm)*	*Total No. of Apertures*
Essex, UK *(type)* (Pennick & Clarke 1972)	Plate	0.5 - 0.65 ($\bar{x}$ =0.5)	0.3 - 0.4 ($\bar{x}$ =0.24)	No	0.6 - 0.9 x 0.4 - 0.8 (n=5)	30 - 40 (n=5)
Cambridge, UK (Preisig & Hibberd, 1982b)	Plate	0.5 - 1.1	0.35 - 0.45	No	0.5 - 1.1 x 0.4 - 0.8	20 - 25 (n=2)
Southhampton, UK (Tong, 1997)	Crown	0.6 (n=1)	0.3 (n=1)	No	0.6 x 0.4 (n=3)	11 - 14 (n=4)
Finland (Vørs, 1992)	Plate	0.7	0.3 (n=1)	No	0.45 - 0.6 x 0.3 - 0.45 ($\bar{x}$ =0.5 x 0.3; n=4)	ND
Denmark (Thomsen, 1975)	Plate	0.6 (n=1)	0.3 (n=1)	No	0.8 - 1.4 x 0.6 - 1.05 ($\bar{x}$ =1.1 x 0.81; n=9)	25 - 50 (n=4)
Mediterranean Sea (Leadbeater, 1974)	Plate	c. 0.45 (n=1)	0.25 (n=1)	Yes	0.6 x 0.4 (n=2)	c. 100 (n=2)
US (Caron, 1999)	Plate	-	-	No	0.85 - 1.1 x 0.6 - 1.1 ($\bar{x}$ =0.98 x 0.83; n=3)	13 - 30 (n=2)
New Zealand (Moestrup, 1979)	Crown	c. 0.6	0.3 (n=1)	Yes	0.5 - 0.6 x 0.4 - 0.5 ($\bar{x}$ =0.6 x 0.4; n=3)	13 - 20 (n=2)
Victoria, Australia (Beech, 1983)	Plate	c. 0.5 (n=1)	0.3 (n=1)	Yes	0.65 - 0.75 x 0.45 0- 0.5 ($\bar{x}$ =0.7 x 0.5; n=4)	c. 100 (n=2)
Tasmania, Australia	Plate	0.3 - 0.4 x 0.4 - 0.65 ($\bar{x}$ =0.6 x 0.4; n=5)	0.3 (n=1)	Yes [1]	0.45 - 0.7 x 0.3 - 0.6 [2] ($\bar{x}$ =0.6 x 0.5; n=24)	26 - 36 [2] (n=10)

ND = Not determined

[1] Protrusions also seen on distal ring and supporting struts.

[2] Measurements recorded for most common form of plate scale only.

***Paraphysomonas foraminifera* Lucas** **Fig. 3.20**

Micrographs: Lucas, 1967; Plate 1C.
Leadbeater, 1972b; Fig. 15.
Thomsen, 1975; Figs. 1 - 5.
Vørs, 1992; Fig. 30 (d).
Tong, 1997; Figs. 14 (e) and (h).

Present Findings.
Scales were found in GSe and ML enrichment cultures established from Little Swanport samples, and ML enrichment cultures from Honey Moon Bay.

This is the first report of this species from Australia, and from the southern hemisphere.

Description.
Scales consisted of a perforated base plate, 0.8 - 0.9 μm in diameter ($\bar{x}$=0.88 μm; n=6), and a long tapering central spine, 1.6 - 1.9 μm in length ($\bar{x}$=1.8 mm; n=5). Plate perforations were arranged in 7 - 8 concentric rings with two distinct, non-perforated bands, one at the scale circumference and the other approximately halfway towards the centre of the scale (Fig. 3.20). These scales matched those described in the original species description (Lucas, 1967).

Variation in spine length is common (Table 3.9), with spines less than 0.5 μm being recorded from Finland, Greenland and the UK (Vørs, 1992; Vørs, 1993; Tong, 1997), and spines over 4 μm found in Danish coastal waters (Thomsen, 1975). A smaller scale was observed in a Storm Bay enrichment culture, with a spine length of only 0.41 μm.

In scales with shorter spines, it was usually difficult to differentiate the intermediate non-perforated band on the scale base (Vørs, 1992, Fig. 30 (d); Tong, 1997, Fig. 14 (h)). These short spines did not always taper, except at the very tip.

Size variability in scales from wild samples was greater than that in cultured material (Table 3.9).

Distribution.

P. foraminifera has been previously reported from temperate to polar coastal waters in the northern hemisphere, including those of Italy, UK, Yugoslavia, Algeria, Norway, Denmark, Finland, Greenland and Canada (Thomsen, 1975, and references therein; Vørs, 1992; Smith and Hobson, 1994; Tong, 1997).

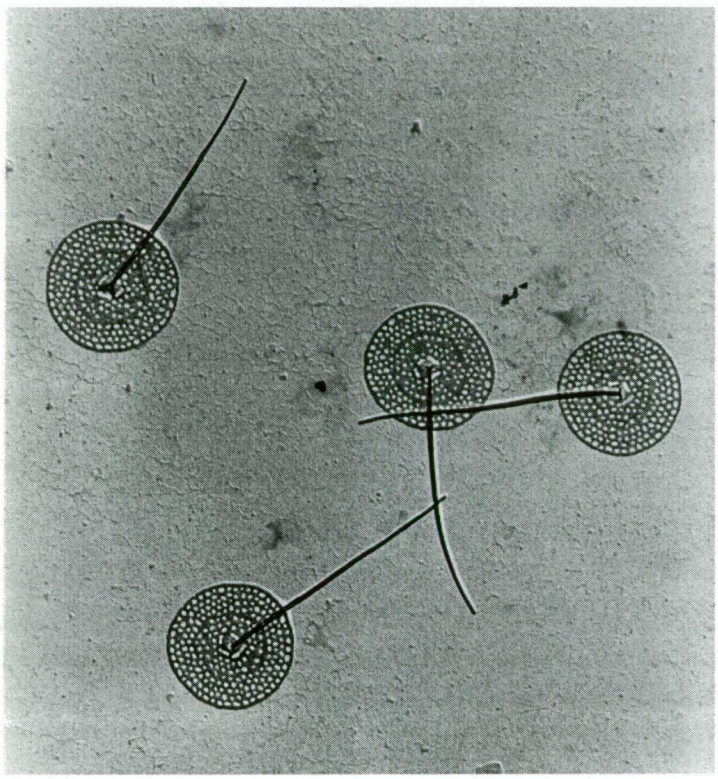


Fig. 3.20: *P. foraminifera* scales (0.8 μm diam.); from a Little Swanport enrichment culture

(Micrograph no: 5313)

Table 3.9: *Paraphysomonas foramifera* spine scales from different locations.

SOURCE	MATERIAL EXAMINED	SPINE LENGTH (μm)	BASE DIAMETER (μm)
Italy (Lucas, 1967)	Culture	1.46 - 1.63 ($\bar{x}$ =1.5)	0.97 - 1.12 ($\bar{x}$ =1.0)
Norway (Leadbeater, 1972b)	Wild	1.2	0.8
Denmark (Thomsen, 1975)	Wild	0.9 - 4.2 ($\bar{x}$ =2.32; n=14)	0.7 - 1.4 ($\bar{x}$ =0.96; n=14)
Finland (Vørs, 1992)	Wild	0.56 (n=2)	0.75 - 0.85 (n=2)
Arctic (Vørs, 1993)	Wild	0.65	ND
UK (Tong, 1997)	Wild	0.45 - 1.3	0.6 - 0.7
Tasmania, Australia - Little Swanport	Culture	1.6 - 1.9 ($\bar{x}$ =1.8; n=5)	0.8 - 0.9 ($\bar{x}$ =0.88; n=6)
- Storm Bay	Culture	0.41 (n=1)	0.7 (n=1)

***Paraphysomonas imperforata* Lucas** **Figs. 3.21 - 3.26**

Micrographs: Lucas, 1967; Plate 1 A - F.
Thomsen, 1975; Figs. 6 - 10.
Takahashi, 1976; Figs. 18 - 19.
Moestrup, 1979; Fig. 4.
Preisig and Hibberd, 1982a; Fig. 2A - F.
Beech, 1983; Plate 2.19 C - D.
Hallegraeff, 1983; Fig. 3.
Vørs, 1992; Fig. 30 (f) - (g).
Santos and Leedale, 1993; Fig. 35.
Tong, 1997; Fig. 14 (d).
Caron et al, 1999. Fig. 1C- D.

Present Findings.
Whole cells or scales were found in nearly every inshore water sample examined, the exceptions being those collected from South Cape and Spring Beach.

P. imperforata grew in enrichment cultures derived from Simmons Point (GSe medium), Southport (ML medium), Little Swanport (ML and GSe/10 media), Pirates Bay (f-Si/100 medium) and Derwent River (GSe/2 and GSe/10 media) samples. Unialgal cultures were established by isolating single cells, but cultures did not survive longer than three months.

Description.
Cells were 2 - 4.5 µm in size, with a long flagellum of 6 - 10 µm ($\bar{x}$=8 µm; n=5) and a short flagellum of 2 - 3 µm (n=2). Flagellar hairs were seen on the long flagellum and ranged from 1.3 - 1.5 µm in length ($\bar{x}$=1.4 µm; n=6) (Fig. 3.21).

Cell size and flagella lengths were slightly smaller than those given by Lucas (1967) in the species description, and were also in the lower range of the sizes compiled by Preisig and Hibberd (1982b) (Table 3.10).

Cells were covered by only one type of scale, which consisted of a flat circular baseplate with a central spine. Three forms of this scale type were observed, with differing structure and spine length.

The most common scales had a spine length ranging from 0.6 - 2.0 μm ($\bar{x}$=1.0μm; n=45), which was equal to, or greater than, the base diameter of 0.6 - 1.0 μm ($\bar{x}$=0.7 μm; n=40). Some spines had a clearly distiguishable tip, at least a third of the total spine length (Figs. 3.22, 3.23), whereas this was not so marked in other spines (Fig. 3.24).

The second form of spine scale had a central annular depression, more pronounced in some scales than others (Fig. 3.25). These scales were similar to those described from Victorian coastal waters (Beech, 1983) and from the East Australian Current (Hallegraeff, 1983) (Table 3.10).

The third form had a spine length ranging from 0.5 - 0.7 μm ($\bar{x}$=0.56 μm; n=8), which was similar to the base diameter of 0.6 - 0.7 μm ($\bar{x}$=0.68 μm; n=12). The spine tip was again at least a third of the total spine length, but was not always easily differentiated (Fig. 3.26). These scales were seen only occasionally and only in wild samples.

Scale dimensions of the Tasmanian material showed greater variability than those given in the type description (Lucas, 1967), but similar average values for spine length and base diameter were found. Records from marine and freshwater environments demonstrate an enormous range of scale size (Table 3.10), and consequently there may well be more than one species.

Preisig and Hibberd (1982a) commented that freshwater forms of *P. imperforata* have very short, pointed spine tips (Figs. 2C, F) while marine forms have longer tips. Spine scales from marine samples in the present study and others (e.g. Vørs, 1992; Tong, 1997) have agreed with these descriptions, supporting the theory that freshwater and marine forms of *P. imperforata* are actually different species.

P. imperforata spine scales are similar to those of *P. antarctica* (as previously discussed), and *P. vestita*. However, *P. vestita* scales have a distinct rim and, either a truncated, or a thin tapering spine (Preisig and Hibberd, 1982a). Despite similar scale morphologies, *P. vestita* is genetically distinct from *P. imperforata* (Caron et al, 1990).

Distribution.

P. imperforata is a common and widely distributed species in both coastal waters and freshwater habitats world-wide (Preisig and Hibberd, 1982b, and references therein; Vørs et al, 1990).

In Australia, *P. imperforata* has been reported from Hobsons Bay and Waratah Bay, Victoria (Beech, 1983), from the East Australian Current (inshore and offshore waters) (Hallegraeff, 1983), and from Tasmanian freshwater lakes (Croome and Tyler, 1985).

In this study, *P. imperforata* was one of the most common species observed, being found at 16 of the 21 sampling sites.

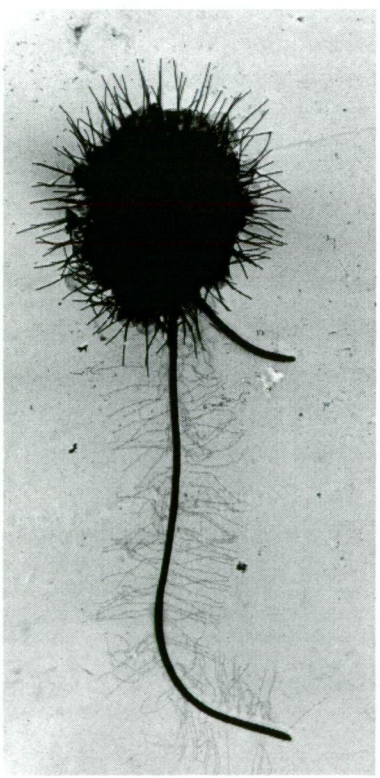


Fig. 3.21: *P. imperforata* cell (c. 0.3 μm); from the Derwent River

(Micrograph no: 5148)

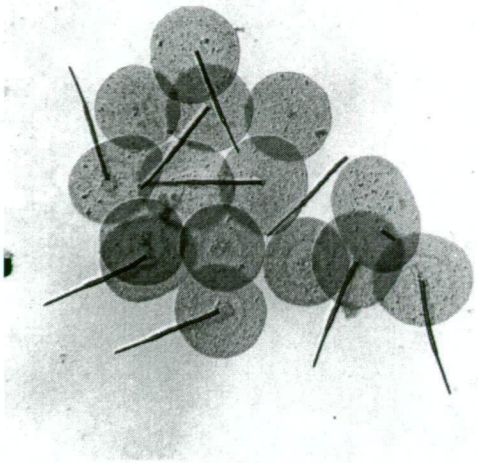


Fig. 3.22: *P. imperforata* long spine scales (1 μm); from Deep Bay

(Micrograph no: 4998)

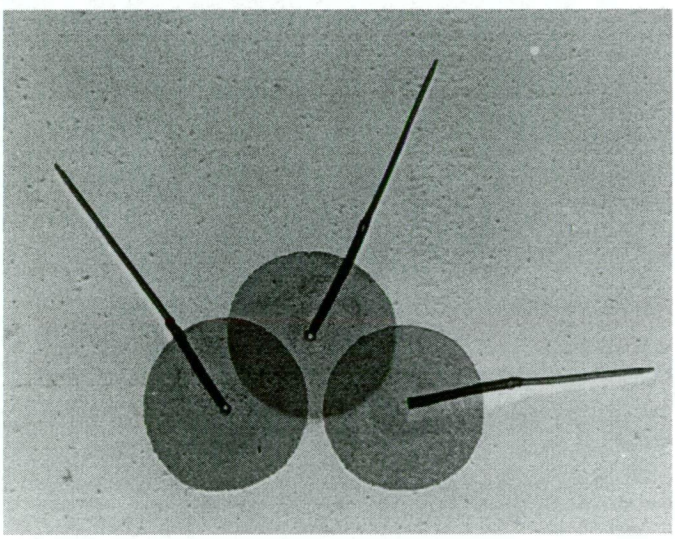


Fig. 3.23: *P. imperforata* scales (2 μm) with well-defined long tips; from a Derwent River enrichment culture

(Micrograph no: 5592)

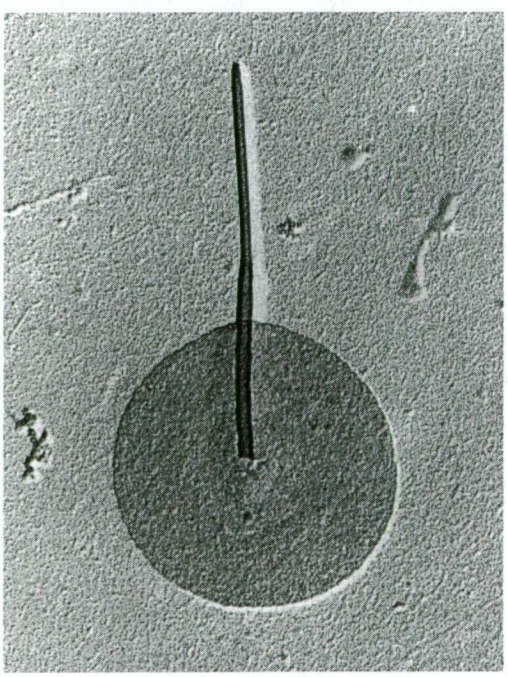


Fig. 3.24: *P. imperforata* scales (1 μm) with indistinct tips; from the Derwent River

(Micrograph no: 4830)

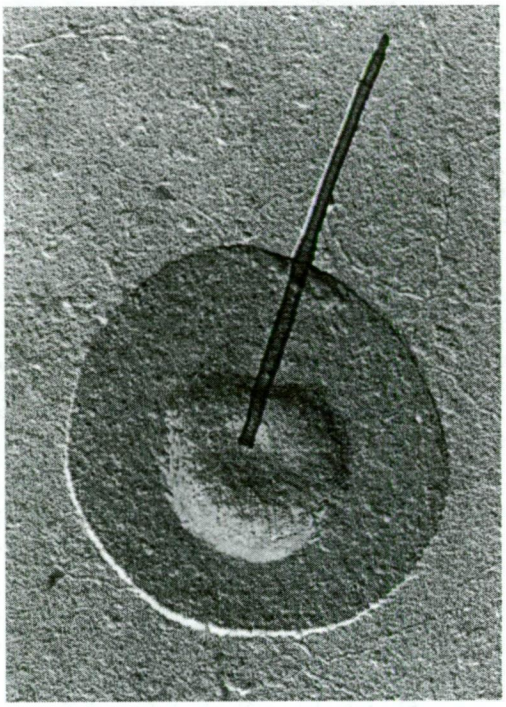


Fig. 3.25: *P. imperforata* spine scale (0.9 μm) with central annular depression; from the Derwent River

(Micrograph no: 4723)

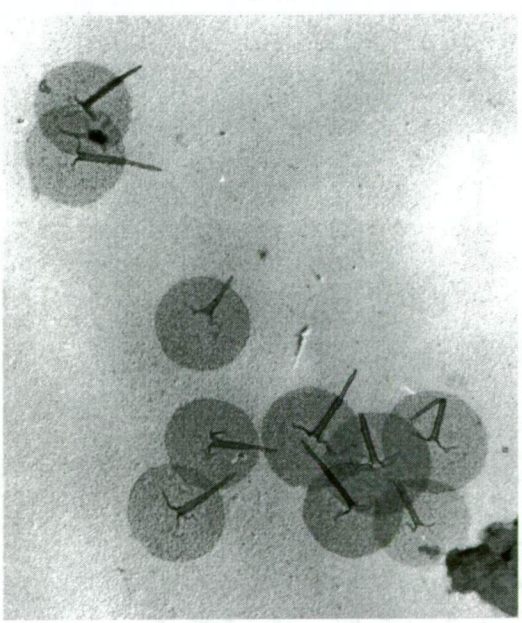


Fig. 3.26: *P. imperforata* short spine scales (0.5 - 0.6 μm); from Fleurty Point

(Micrograph no: 4959)

Table 3.10: Comparison of *Paraphysomonas imperforata* from overseas and Australian waters.

SOURCE	MATERIAL EXAMINED	CELL DIAM.	FLAGELLA		FLAGELLAR HAIRS	SPINE SCALES	
			Long	*Short*		*Spine Length*	*Base Diam.*
		(µm)	*(µm)*	*(µm)*	*(µm)*	*(µm)*	*(µm)*
English Channel (Lucas, 1967)	Marine	3.8 - 5.1 ($\bar{x}$ =4.5)	10 - 20	4 - 5	1.5	0.88 - 1.1 ($\bar{x}$ =1.0)	0.7 - 0.85 ($\bar{x}$ =0.7)
UK (Preisig & Hibberd, 1982b)	Marine, Freshwater	1.7 - 18	5.5 - 51	2.8 - 5.1	1.5 - 2.3	0.8 - 25 .0	0.4 - 3.3
Victoria, Australia (Beech, 1983)	Marine	c. 4.5 (n=1)	9 (n=1)	3 (n=1)	2.3 - 2.5 (n=3)	1.2 - 1.5 ($\bar{x}$ =1.3; n=3)	0.7 (n=3)
East Australian Current (Hallegraeff, 1983)	Marine	-	-	-	-	1.6	0.9
Southampton, UK (Tong, 1997)	Marine	2.5 - 5	ND	ND	ND	0.6 - 1.65	0.55 - 0.65
Tasmania, Australia	Marine	2.1 - 4.5 ($\bar{x}$ =3; n=8)	6 - 10 (n=5)	2 - 3 (n=2)	1.3 - 1.5 ($\bar{x}$ =1.4; n=6)	0.6 - 2.0 [1] ($\bar{x}$ =1.3, n=45)	0.6 - 1.0 [1] ($\bar{x}$ =0.7, n=40)

ND = Not determined

[1] Measurements recorded for the most common scale form only.

***Paraphysomonas* cf. *takahashii* Cronberg et Kristiansen** **Figs. 3.25 - 3.27**

Micrographs: Cronberg and Kristiansen, 1980; Fig. 10B - D.
Thomsen et al, 1981; Figs. 49 - 56.
Preisig and Hibberd, 1982a; Fig. 3.
Vørs, 1992: Fig. 31 (f) - (g).

Present Findings.
Whole cells and numerous scales were found in Derwent River samples. Scales were also recorded from Dru Point and Southport.

This is a new record for Australian coastal waters.

Description.
These distinctive scales resembled those of *P. takahashii.* However, there were several differences in cell size, scale structure and habitat.

Cells were approximately 3 μm in size, which was marginally smaller than the average cell size (c. 5 μm) recorded for *P. takahashii.* Flagellar lengths also differed from previous records. The long flagellum, 7 μm (n=1), was less than half the given average length, whereas the short flagellum, 3 μm (n=1), was of similar length to that of *P. takahashii.* Flagellar hairs, ranging from 1.4 - 2.3 μm, were observed on the long flagellum (Fig. 3.25), and were slightly longer than those previously recorded (Table 3.11).

Scales had a circular or elliptical base, 0.9 - 1.3 x 0.7 - 1.0 μm ($\bar{x}$=1.1 x 0.8 μm; n=7), with 60 - 130 perforations arranged in 4 - 6 concentric rings. A central spine, 0.8 - 2.2 μm in length ($\bar{x}$=1.2 μm; n=10), was attached to this meshwork base by diverging struts (Figs. 3.26, 3.27). Unlike the long pointed spines of *P. takahashii,* this spine was shorter and had a distinctly truncated tip.

P. takahashii is described as "a polymorphic species with two types of meshwork scale" (Preisig and Hibberd, 1982a). One type has a long tapered spine, while the other has only a short vestigal spine (Preisig and Hibberd, 1982a; Fig. 3). Both scale types are usually present on the one cell (Thomsen et al, 1981, Figs. 53 and 54; Preisig and Hibberd, 1982a, Fig. 3A), but only scales with long spines were observed in the Tasmanian material.

There are many variations of these two basic scale types. For example, the number of perforations in the meshwork base can vary from 30 large apertures to several hundred small holes, which may be arranged in 2 - 10 concentric rings (Nicholls, 1981, Fig. 19; Preisig and Hibberd, 1982a, Figs. 3C, D). The number of spines found on a single meshwork base can also vary; two spines on the one base have been reported (Thomsen et al, 1981; Figs. 50, 57). Alternatively, the spine may be completely absent (Nicholls, 1981; Fig. 16), or replaced by an accumulation of scale material (Preisig and Hibberd, 1982a; Figs. 3E, F, I).

Given this variability in scale structure, the scales observed in this study could represent a previously undescribed form of *P. takahashii*, or possibly a new species of *Paraphysomonas*.

Distribution.

P. takahashii has been reported only rarely, and mostly from temperate freshwater habitats (Preisig and Hibberd, 1982a, and references therein). There has been only one other report from the marine environment; a few scales were found in samples collected from the Gulf of Finland (Vørs, 1992).

At least three other species of *Paraphysomonas* have been previously recorded from both marine and freshwater environments (Presig et al, 1991), and *P. takahashii* may also have a broad salinity tolerance.

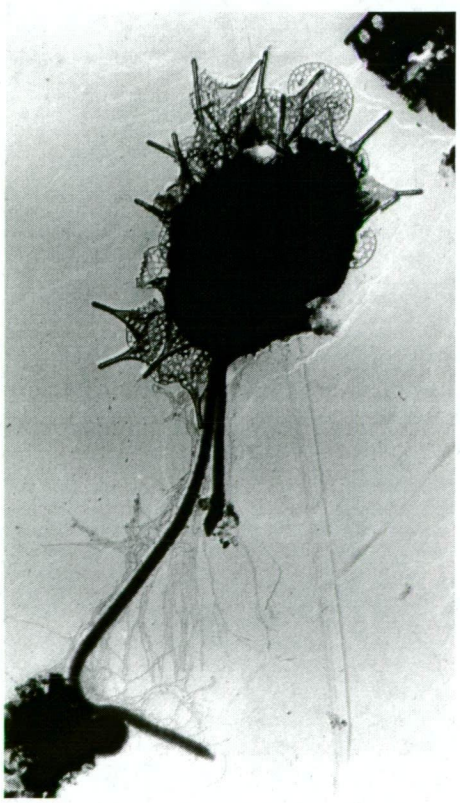


Fig. 3.25: *P.* cf. *takahashii* cell (c. 3 μm); from the Derwent River

(Micrograph no: 4637)

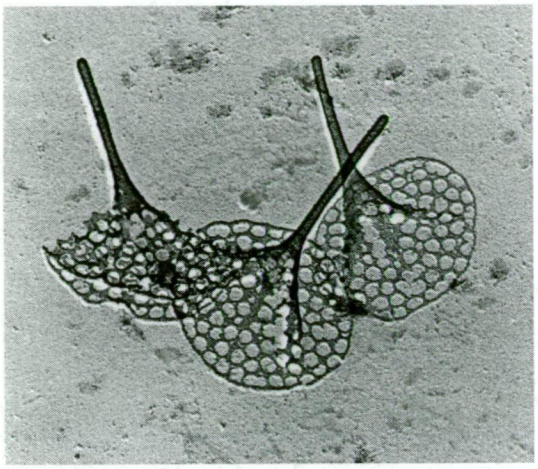


Fig. 3.26: *P.* cf. *takahashii* spine scales (1.5 μm); from the Derwent River

(Micrograph no: 4757)

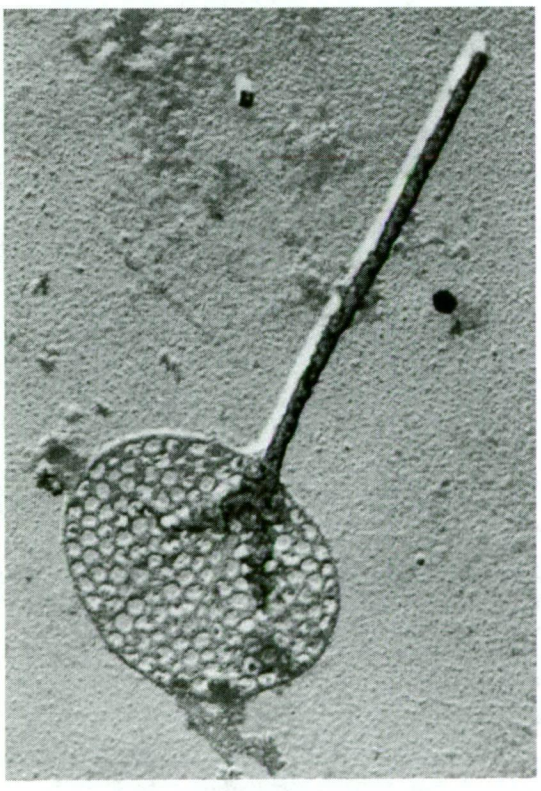


Fig. 3.27: *P.* cf. *takahashii* long spine scale (2 μm); from the Derwent River

(Micrograph no: 4678)

Table 3.11: Comparison of *Paraphysomonas takahashii* and *Paraphysomonas* cf. *takahashii*.

SPECIES	CELL DIMENSIONS (µm)	FLAGELLA		FLAGELLAR HAIRS (µm)	SPINE SCALES	
		Long (µm)	*Short* (µm)		*Base Dimensions* (µm)	*Length* (µm)
P. takahashii [1]	3.5 - 9.0	25 - 36	3.0 - 5.5	1.4 - 1.7	1.2 - 3.0 x 0.9 - 2.1	1.5 - 8.2 0.1 - 0.8 [2]
P. cf *takahashii* (Tasmanian material)	c. 3	7 (n=1)	3.0 (n=1)	1.4 - 2.3 ($\bar{x}$ =1.8; n=6)	0.9 - 1.3 x 0.7 - 1.0 ($\bar{x}$ =1.1 x 0.8; n=7)	0.8 - 2.2 ($\bar{x}$ =1.2; n=10)

[1] From previous records: Preisig & Hibberd, 1982a, and references therein; Vørs 1992

[2] Lengths of short spine scales

Paraphysomonas sp. 1 Figs. 3.28 - 3.29

Present Findings.

Scales were found in Derwent River samples and in ML enrichment cultures derived from the Storm Bay. Unfortunately, unialgal cultures were not established.

Description.

Scales had a round to oval base plate with a central perforation. An oval structure of 8 - 10 arches, connected with an elaborate "lacework" apical ring, surrounded this central perforation (Figs. 3.28, 3.29). The base plate ranged from 0.6 - 0.65 x 0.4 - 0.5 μm ($\bar{x}$=0.6 x 0.45 μm; n=4) in size, and the height of the arched structure was approximately 0.2 μm.

Paraphysomonas scales with this structure have not been described from either marine or freshwater habitats. The scales recorded in this study probably represent a new marine *Paraphysomonas* species, but more complete material is required.

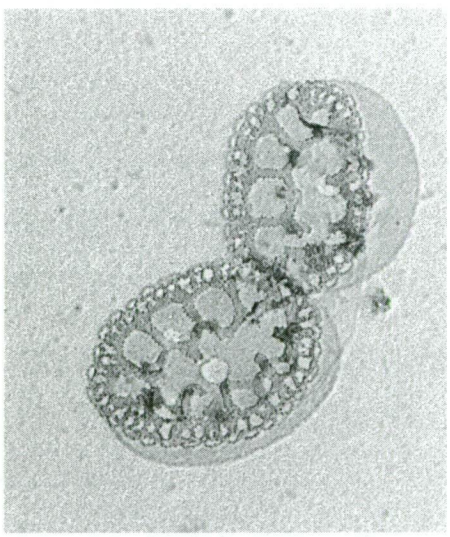


Fig. 3.28: *Paraphysomonas* sp. 1, scales (0.6 x 0.5 μm) with elaborate "lacework" apical ring - distal view; from a Derwent River enrichment culture

(Micrograph no: 5565)

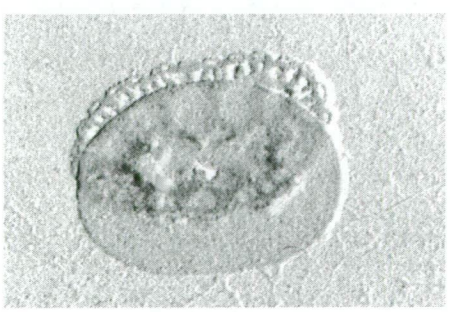


Fig. 3.29: *Paraphysomonas* sp. 1, scales - proximal view; from the Derwent River

(Micrograph no: 4696)

***Paraphysomonas* sp. 2** **Fig. 3.30**

Present Findings.

A field of scales was found in a Derwent River sample.

Description.

Scales were elliptical, c. 0.4 x 0.3 μm, with numerous concentrically-arranged perforations and a marginal patternless band, c. 0.02 μm in width.

Scales had a superficial resemblance to those of *Paraphysomonas punctata* which also have perforated surfaces and patternless margins (Presig and Hibberd, 1982b). However, perforations are usually arranged in rows, rather than concentrically, and scales are at least three time larger than those reported in this study. *P. punctata* is a common freshwater species and has not been recorded from the marine environment.

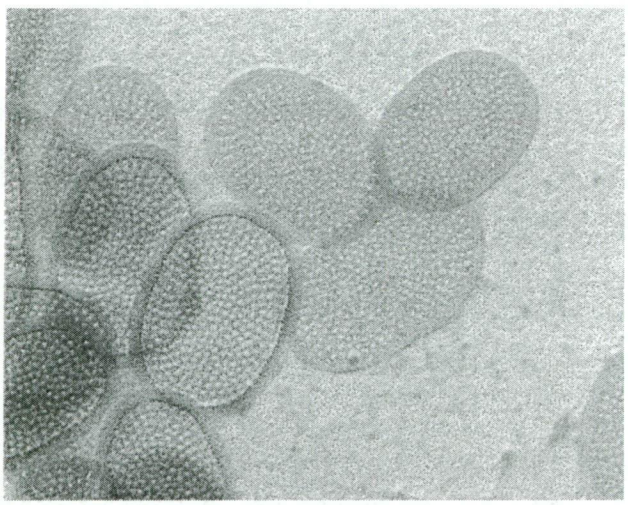


Fig. 3.30: *Paraphysomonas* sp. 2, field of scales (c. 0.4 x - 0.3 μm); from the Derwent River

(Micrograph no: 5590)

***Petasaria heterolepis* Moestrup** **Fig. 3.31**

Micrographs: Moestrup, 1979; Figs. 61 - 66.

This unique uniflagellate species is known only from whole mount preparations and may or may not have chloroplasts (Patterson and Zölffel, 1991). Its taxonomic affiliation is uncertain.

Present Findings.
Scales were found in Derwent River, Pipeclay Lagoon and Eaglehawk Neck samples.

This is a new record for Australian waters.

Description.
Two types of scales were distinguished (Fig. 3.31). Large "hat-like" scales were 1.5 -1.8 x 1.6 - 1.9 μm in size (n=2), partially perforated, with a raised rim and a central projection. Smaller, circular "spiderweb" scales were 0.35 - 0.42 μm in diameter ($\bar{x}$=0.4 μm; n=4), with eight evenly-spaced "spokes" radiating from the centre to the scale edge.

Scale structure was similar to that observed by Moestrup (1979). However, the larger scales from the Tasmanian material were generally smaller than those from New Zealand (Table 3.12).

Distribution.
P. heterolepis was originally described from New Zealand, with later reports from the Red Sea and Danish coastal waters (Moestrup, 1979), indicating that this species has a potentially wide distribution.

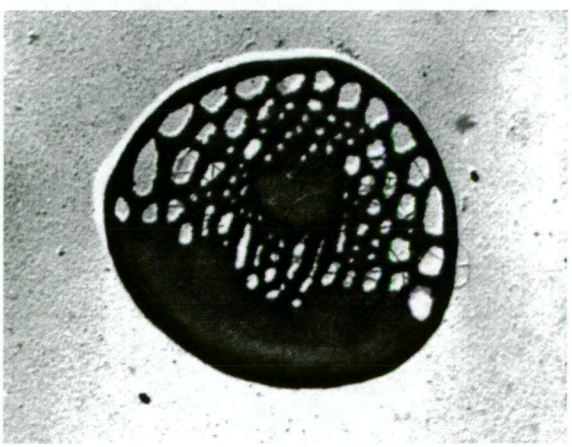


Fig. 3.31: *P. heterolepis* scales, smaller "spiderweb" scales are obscured by the larger scale; from Pipeclay Lagoon

(Micrograph no: 5028)

Table 3.12: Size of *Petasaria heterolepis* scales from New Zealand and Tasmania.

SOURCE	"HAT-LIKE" SCALES		"SPIDERWEB" SCALES
	Length (µm)	*Width (µm)*	*(µm)*
New Zealand (Moestrup, 1979)	1.7 - 2.9	1.9 - 3.2	c. 0.45
Tasmania	1.5 - 1.8 (n=2)	1.6 - 1.9 (n=2)	0.35 - 0.42 ($\bar{x}$ =0.4; n=4)

***Thaumatomastix salina* (Birch-Andersen) Beech et Moestrup** **Fig. 3.32**

Synonyms: ***Chrysosphaerella salina* Birch-Andersen**
***Spiniferomonas salina* (Birch-Andersen) Nicholls**

Micrographs: Moestrup, 1979; Figs. 5 and 7.
Beech, 1983; Plate 2.18 A - F.
Takahashi and Hara, 1984; Figs. 13 - 18.
Beech and Moestrup, 1986; Figs. 4 - 14.
Vørs, 1992; Fig. 46 (a) - (c).
Thomsen et al, 1993; Figs. 37 - 41.
Tong, 1997; Figs. 20 (d) - (f); 21 (f).

The phylogenetic position of *Thaumatomastix* is uncertain. Previous authors have placed this genus in the classes Chrysophyceae and Raphidophyceae (Beech and Moestrup, 1986). It has been recently referred to in the literature as a heterotrophic protist belonging to "Thaumatomastigaceae, Protista incertae sedia" (Thomsen et al, 1993; Tong, 1997).

Present Findings.
Isolated plate scales were commonly found in Derwent River and Pipeclay Lagoon samples. They were also found at all D'Entrecastreaux Channel sites, in addition to Deep Bay, Southport, Roches Beach and Little Swanport. However, no whole cells, spine or flagellar scales were identified from any of these samples.

T. salina did not grow in any enrichment media used. Addition of organic compounds to the media may have promoted growth as *T. salina* is a heterotrophic species. Beech and Moestrup (1986) reported that cell numbers increased when water samples were left in the dark for several days.

Description.
This species was identified by its characteristic plate scales, described as consisting of two fused plates with the distal dish-like plate centrally attached to the flat proximal plate (Beech and Moestrup, 1986; Fig. 9).

Scales were elliptical, 1.4 - 1.5 x 0.95 - 1.0 µm in size ($\bar{x}$=1.4 x 0.96 µm; n=5). They were divided into two semi-elliptical areas by a central band crossing the width, and had a broad outer rim, 0.12 - 0.18 µm in width ($\bar{x}$=0.15 µm; n=5) (Fig. 3.32). Each semi-elliptical area contained 15 - 20 regularly-arranged perforations. This figure

differed from previous reports, which specified either a varying number of perforations, or the complete absence of perforations (Beech, 1983; Takahashi and Hara, 1984; Beech and Moestrup, 1986). Scale dimensions agreed with measurements made by other investigators (Table 3.13).

Distribution.

T. salina has been previously reported from temperate waters in both hemispheres, including those of: Denmark, Finland, Japan, UK, Canada, New Zealand and south-east Australia (Beech and Moestrup, 1986 and references therein; Smith and Hobson, 1994; Tong, 1997). It has been found at a wide range of temperatures (4 - 22°C) and salinities (3 - 35 psu).

In this survey, *T. salina* was a common species, recorded from 10 of the 21 sampling sites, and at temperatures ranging from 10 - 20 °C.

Fig. 3.32: *T. salina* plate scale (1.4 x 1.0 μm); from the Derwent River

(Micrograph no: 4796)

Table 3.13: *Thaumatomastix salina* plate scales from different locations.

SOURCE	MATERIAL EXAMINED	PLATE SCALE DIMENSIONS *(μm)*
Victoria, Australia (Beech & Mostrup, 1986)	Wild, Enrichment	1.35 - 1.45 x 8 - 9 (n=2)
Finland (Vørs, 1992)	Wild	1.3 x 0.8 (n=2)
UK (Tong, 1997)	Wild, Enrichment	1.5 x 0.5 (n=1)
Tasmania, Australia	Wild	1.4 - 1.5 x 0.95 - 1.0 ($\bar{x}$ =1.4 x 0.96; n=5).

***Thaumatomastix* cf. *thomseni* Tong** **Figs. 3.33 - 3.34**

Micrographs: Tong, 1997; Fig. 22 (a) - (c).

Present Findings.
Scales were found in Roches Beach and Fleurty Point samples.

This is a new record for Australian waters.

Description.
These elliptical scales consisted of two partially fused plates with an longitudinal stripe (Fig. 3.33). The upper structure appeared fragile, as seen in the distorted scales in Fig. 3.34. Scales were 0.7 - 0.8 µm in length and approximately 0.5 µm in width (n=6).

Scales resembled plate scales of *T. thomseni* recently described by Tong (1997). However, the fine striations on either side of, and parallel to, the central longitudinal stripe were not seen in the Tasmanian material, nor were small flagellar scales observed. Unfortunately, whole cells were not found in the Tasmanian samples for further comparison.

T. thomseni differs from other *Thaumatomastix* species in that it lacks spine scales, and its plate scales have a characteristic central longitudinal stripe (Tong, 1997).

Distribution.
T. thomseni has been previously reported from Denmark and the UK (Tong, 1997, and references therein).

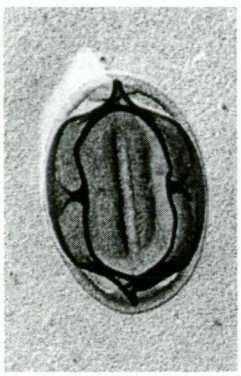


Fig. 3.33: *T.* cf. *thomseni* plate scale (0.7 x 0.5 μm); from Fleurty Point

(Micrograph no: 4958)

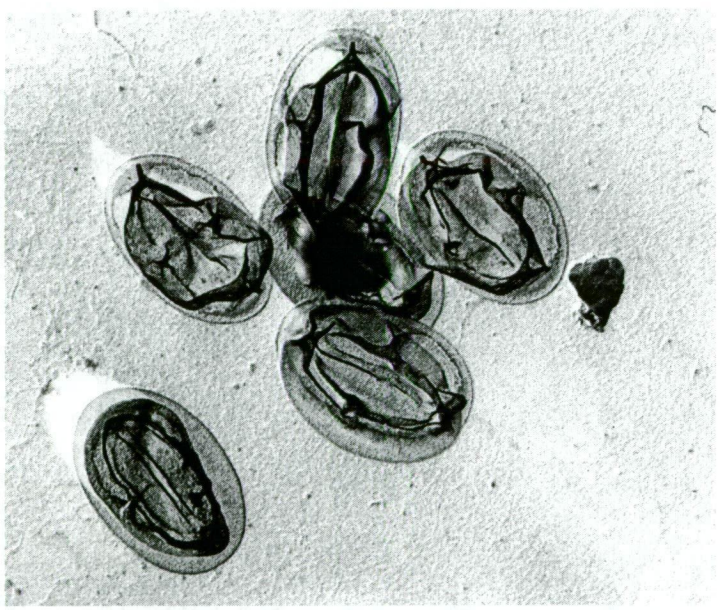


Fig. 3.34: *T.* cf. *thomseni* plate scales showing collapsed structure; from Fleurty Point

(Micrograph no: 4968)

***Thaumatomastix tripus* (Takahashi et Hara) Beech et Moestrup**

Figs. 3.35 - 3.37

Synonyms: ***Chrysosphaerella tripus* Takahashi and Hara**
***Spiniferomonas salina* (Takahashi and Hara) Nicholls**

Micrographs: Beech and Moestrup, 1986; Figs. 23 - 25.

Location.
Whole cells were seen in the Southport sample. Scales were more common, being found in samples from the Derwent River, Dru Point, Deep Bay, Pipeclay Lagoon, Eaglehawk Neck and Little Swanport.

Like *Thaumatomastix salina*, *T. tripus* did not grow in any of the enrichment media used. *T. tripus* has been reported to bloom in water samples after collection, but these blooms were short-lived, lasting for less than 24 hours (Beech and Moestrup, 1986).

Description.
Whole cells of *T. tripus* were 10 - 16 μm in diameter (n=2). One specimen retained its long flagellum, 21 μm in length, but the short flagellum was not seen. Cells were covered with 20 - 30 spine scales radiating from the cell surface, and numerous triangular scales (Fig. 3.35).

Spine scales were thin and slightly curved, ranging in length from 8 -16 μm ($\bar{x}$=12 μm; n=8), with distinct three-pointed tips (Fig. 3.36). The proximal end of each scale consisted of a round base plate, 1.0 μm diameter (n=2), above which there was a smaller distal disc, 0.4 μm diameter (n=2). This was the point where three individual struts arising from the base plate fused (Beech and Moestrup, 1986). The distance between the base plate and the distal disc was 0.6 μm (n=2).

Triangular scales were 1.0 - 2.6 μm in length ($\bar{x}$=1.6 μm; n=13) with a small central perforation and an outer rim, 0.3 μm in width (n=2) (Fig. 3.37). These scales are formed by two triangular plates joined by three "feet" (Beech and Moestrup, 1986).

Measurements of scale dimensions agreed with previous reports (Table 3.14).

Distribution.

T. tripus has been previously reported from Japan, Denmark, South Africa, Canada and south-east Australia, at salinities ranging from 15 - 35 psu (Takahashi and Hara, 1984; Beech and Moestrup, 1986; Smith and Hobson, 1994).

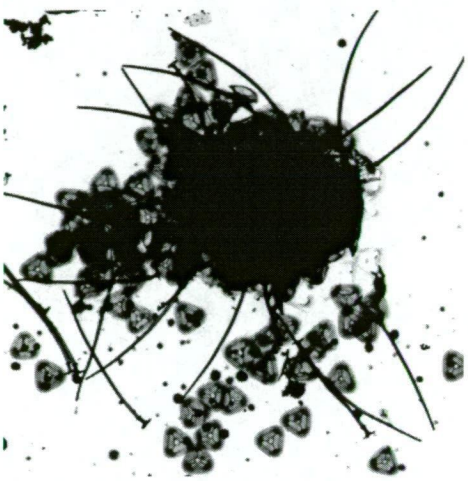


Fig. 3.35: *T. tripus* cell (10 μm) with plate and spine scales; from the Derwent River

(Micrograph no: 5578)

Fig. 3.36: *T. tripus* spine scale (9 μm) showing three-pointed tip, distal disc and basal plate; from the Derwent River

(Micrograph no: 4988)

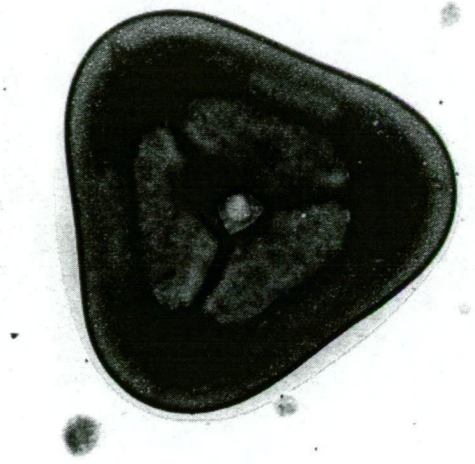


Fig. 3.37: *T. tripus* triangular scale (2 μm); from the Derwent River

(Micrograph no: 5580)

Table 3.14: *Thaumatomastix tripus* scales from different locations.

SOURCE	SPINE SCALES			TRIANGULAR SCALES
	Length (*µm*)	*Base Plate Width* (*µm*)	*Distal Plate Width* (*µm*)	(*µm*)
Japan (Takahashi & Hara, 1984)	7 - 16	0.8 - 1.4	0.3 - 0.6	1.9 - 2.5
South Africa (Beech & Moestrup, 1986)	10	0.9	0.4	1.8 - 2.0
Victoria, Australia (Beech & Moestrup, 1986)	7 - 15	0.9	0.4	1.8 - 1.9
Tasmania, Australia	8 - 16 ($\bar{x}$ =1.2; n=8)	1.0 (n=2)	0.4 (n=2)	1.0 - 2.6 ($\bar{x}$ =1.6; n=13)

***Thaumatomastix* sp. 1** **Fig. 3.38**

Present Findings.
Only one scale of this type was found in a Derwent River sample.

Description.
This scale was similar in shape to the elliptical scale of *T. salina,* having two semi-elliptical areas separated by a central band. However, these areas did not have any of the perforations seen in scales of *T. salina.* Instead, there were a series of arches around the inner perimeter (Fig. 3.38). The scale size was approximately half that of *T. salina* (0.9 x 0.5 μm compared to 1.4 x 1.0 μm), and the outer rim was narrower in width (0.06 μm compared to 0.12 μm).

The "symmetry of three," discussed by Beech and Moestrup (1986) as being typical of the body scales of *T. salina* and *T. tripus*, was also present in this scale structure, as the non-perforated triangular areas within the semi-elliptical sections of the scale.

A similar scale was illustrated by Takahashi and Hara (1984; Fig. 14) from the Seto Inland Sea, Japan.

This material may represent a previously undescribed species of *Thaumatomastix*, but more complete material is required for unambiguous characterisation.

***Thaumatomastix* sp. 2** **Fig. 3.39**

Present Findings.
Only one scale of this type was found in a Derwent River sample.

Description.
This triangular scale (1 μm) was similar to that of *T. tripus*, but lacked the central perforation. There was a triangular thickening where the two plates joined (Fig. 3.39).

This material may also represent a previously undescribed species of *Thaumatomastix*, but again more material is required for characterisation.

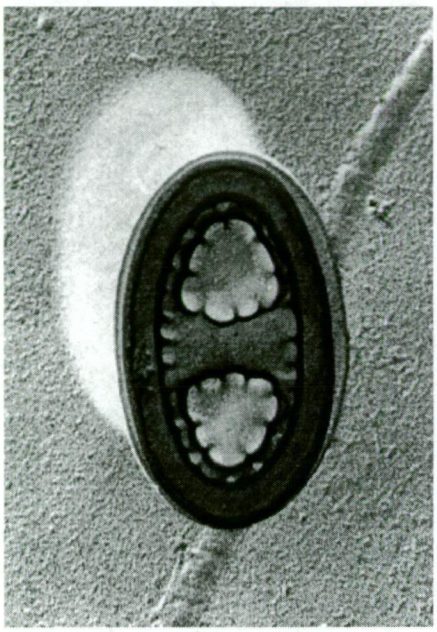


Fig. 3.38: *Thaumatomastix* sp. 1, elliptical scale (2 μm); from the Derwent River

(Micrograph no: 4829)

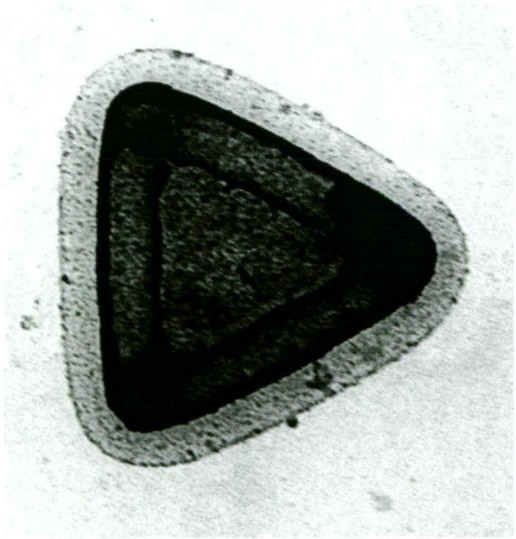


Fig. 3.39: *Thaumatomastix* sp. 2, triangular scale (1 μm); also from the Derwent River

(Micrograph no: 5230)

***Thaumatomastix* sp. 3** **Fig. 3.40**

Present Findings.
Scales were found in several Derwent River samples.

Description.
Scales (c. 0.6 μm) were triangular with a characteristic central "Y" Two struts were found at each apex of the base plate which supported an upper triangular structure as shown in Fig. 3.40.

***Thaumatomastix* sp. 4** **Fig. 3.41**

Present Findings.
Scales were found from Oyster Cove Point.

Description.
These small triangular scales (c. 0.5 μm) were fairly fragile and often collapsed. The triangular base had a row of dots or perforations around its rim, and there appeared to be a circular structure at each apex (Fig. 3.41).

***Thaumatomastix* sp. 5** **Fig. 3.42**

Present Findings.
One scale was found in the Ocean Beach sample.

Description.
This scale (c. 0.4 μm) had a perforated triangular base, above which a slightly smaller triangular plate was rotated through 60°. Three irregular processes from the corners of this upper plate met a central point (Fig. 3.42).

Another undescribed species of *Thaumatomastix* has scales with a similar orientation of triangular plates, but with a much smaller upper plate raised on struts above the base (Thomsen et al, 1993; Tong, 1997). This species has been reported from the Baltic Sea, Denmark and the UK.

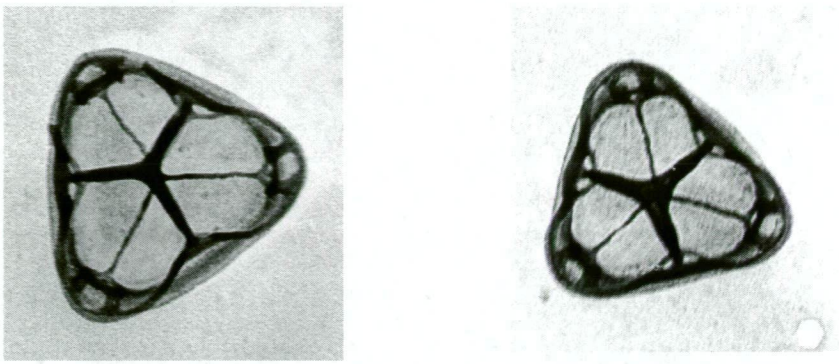


Fig. 3.40: *Thaumatomastix* sp. 3, triangular scales (c. 0.6 μm); from the Derwent River

(Micrographs no: 5226 and 5227)

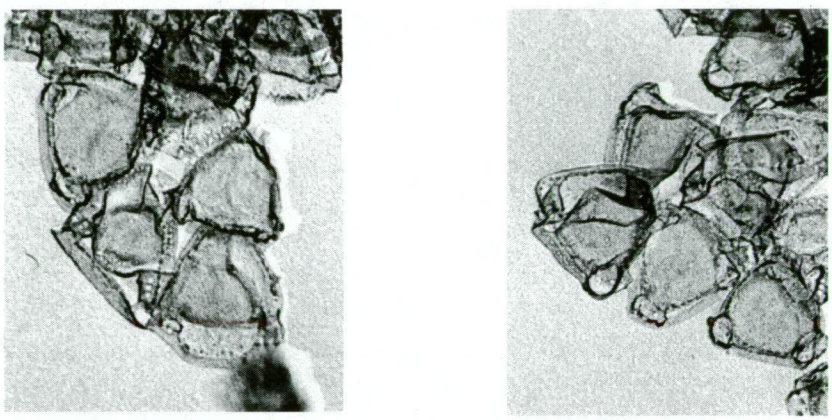


Fig. 3.41: *Thaumatomastix* sp. 4, triangular scales (c. 0.5 μm); from Oyster Cove Point

(Micrograph no: 4953)

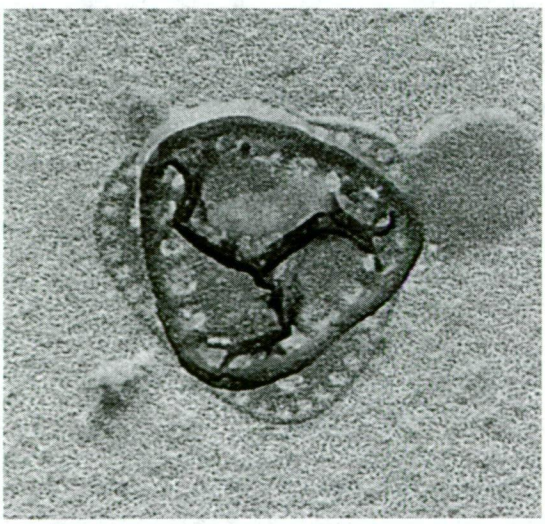


Fig. 3.42: *Thaumatomastix* sp. 5, triangular scale (0.4 μm); from Ocean Beach

(Micrograph no: 5327)

3.4 Discussion

Scale-bearing chrysophytes were common in samples collected in this survey, with most species observed from more than one site, indicating that they are an important component of the marine flora. *Meringosphaera mediterranea*, *Paraphysomonas butcheri* and *P. imperforata* were the most common species, in contrast to *Chrysolepidomonas* cf. *marina*, *P. antarctica* and *P. bandensis*, each of which were reported only once.

Some *Paraphysomonas* species have wide salinity tolerances. *P. butcheri* and *P. imperforata* are common in both marine and freshwater habitats. *P. bandaiensis* and *P. takahashi* are usually freshwater species, but in the present study, they were found in marine waters. However, neither of these species grew in enrichment media with salinity of 28 or 35 psu. *P. antarctica* and *P. foraminfera*, reported only from marine environments, grew well in enrichment culture, as did *P. butcheri* and *P. imperforata.*

Not all species observed grew in enrichment culture. Many of the enrichments prepared included GeO_2 to prevent diatom growth by competitively inhibiting the incorporation of silica into the cell wall (Lewin, 1966; Markham and Hagmeier, 1982). There may be a similiar inhibitory effect for *Paraphysomonas* and other silica-containing chrysophytes. *P. imperforata* grew in a low-nutrient enrichment medium lacking additional silicate (f-Si/100), indicating that there is adequate silicate available for growth in natural seawater.

Species which did grow in enrichment culture were difficult to maintain long term, with only one species, *Chrysolepidomonas* cf. *marina* (CS-490) successfully cultured. Given that several species were heterotrophic, higher concentrations of organic substances in the culture medium may have enhanced growth. Pennick and Clarke (1972) observed that *P. butcheri* grew best in the presence of high bacterial numbers or coccolithophorids, and Lucas (1967) reported that *P. foraminifera* thrived in cultures with a high bacterial load. In the Culture Collection of Algae and Protozoa, UK, *Paraphysomonas* is routinely cultured with the addition of a pea to the culture medium to provide additional nutrients (M. DeVille, pers. comm.).

Examining scale morphology, variation in spine length of *M. mediterranea* and *P. foraminifera* was marked, with spines of *M. mediterranae* ranging from 12 - 40 μm, and those of *P. foraminifera* from 0.5 - 4 μm. Both *P. butcheri* and *P. imperforata* had a range of scale forms, with five different forms of plate scale observed for *P. butcheri*, and 3 forms of spine scale for *P. imperforata.* These species have silicified

scales, and morphological variation may be related to the uptake of silicate from the environment. In the coccolithophorid, *Emiliania huxleyi,* the amount of carbonate available affects coccolith production, with more coccoliths present on cells enriched with either dissolved organic or inorganic carbon (Nimmer and Meret, 1993; Takano et al, 1995). Further investigation is required to determine causes of morphological variation in silica-scaled chrysophytes.

Consistent differences in in scale morphology of freshwater and marine forms of *P. imperforata* has led to the suggestion that there may exist two different species (Preisig and Hibberd, 1982a), and this may extend to other *Paraphysomomas* species found in both marine and freshwater habitats.

Genetic studies have been used to examine species with similar scale morphologies, for example, the spine-bearing species, *P. imperforata*, *P. bandaiensis*, *P. foraminifera* and *P. vestita, which* supported phylogenetic grouping based on scale morphology (Caron et al, 1999). It would be useful to apply genetic techniques to compare populations of the same species with varying scale morphologies.

4. DIVISION HAPTOPHYTA: CLASS PRYMNESIOPHYCEAE (Exclusive of Coccolithophorids)

Most Prymnesiophyceae occur in the marine environment, and constitute a major component of the phytoplankton in both coastal and oceanic waters. These golden-brown algae are either flagellated or produce flagellate cells at some stage in their life history. They have a characteristic haptonema, a unique filamentous organelle usually found in close association with the flagella. The majority of Prymnesiophyceae carry species-specific organic scales on the cell body; those which produce small calcified scales (coccoliths) were not studied in the present work.

Several different classification systems exist for this group and there is much debate about correct taxonomic names (Green and Jordan, 1994; Rhodes, 1994; Edvardsen et al, 2000). Names are either typified, that is based on an existing genus, or descriptive, referring to a physical attribute common to members of the group. Consequently, in the literature, the division is referred to as both the "Prymnesiophyta" and the "Haptophyta", and the class as the "Prymnesiophyceae" and the "Haptophyceae." According to Green and Jordan (1994), the division name "Prymnesiophyta" is not valid, whereas both class names are correct. However, the International Code of Botanical Nomenclature states that each taxonomic group can have only one correct name. In addition, a classification system which confidently reflects relationships between different taxonomic groups has not yet been agreed upon. In the present study, classification from class to genus followed that given in Throndsen (1997).

4.1 Taxonomic Features

Within the Prymnesiophyceae, taxonomic groups are generally distinguished on the basis of their flagella and haptonema, and their scale morphology (Table 4.1).

There are two flagella, which are usually smooth and of approximately equal length. However, in the order Pavlovales (recently differentiated as a separate class by Edvardsen et al, 2000), cells have one long flagellum with fine hairs or tiny "knob" scales, and one short vestigial flagellum which may be visible only under the electron microscope (Green, 1980).

During swimmimg, flagellar motion may be the same (homodynamic) or different (heterodynamic). In some species of *Chrysochromulina*, both flagella beat with an undulating wave motion, while in *Pavlova* species, the long flagellum has a wave-like beat and the short flagellum has a stiff inflexible action.

The haptonema may be long and capable of coiling when relaxed, or short and stiff, and may protrude in the swimming direction. In some prymnesiophyte species, it is very much reduced and only visible under the electron microscope. The function of the haptonema is not fully understood; it is sometimes used for anchoring the cell, and may have a role in phagotrophic feeding of some *Chrysochromulina* species (Inouye and Kawachi, 1994).

Cells may be spherical, round or flattened, elongated or saddle-shaped, and are usually free-living, although some species form colonies. For example, motile "donut-shaped" colonies are seen in *Corymbellus aureus,* and large spherical or irregular-shaped mucilaganeous colonies are common in the genus *Phaeocystis.*

Organic scales covering the cell body are small, and usually distinguishable only under the electron microscope, although large spiny scales or the complete scale covering may be seen with the light microscope.

The basic type of organic scale is generally considered to be an oval, two-layered plate with fibrils arranged radially on the inner face and roughly concentrically on the outer surface. Such simple scales are seen in species of *Isochrysis* and *Prymnesium* (Green and Pienaar, 1977; Green et al, 1982). Variations on this basic scale type result in a wide diversity of structural types, as found in the genus *Chrysoschromulina.* More than one scale type may be present in the same species, and different scales are often arranged in discrete layers on the cell body. Unique "knob" scales occur in *Pavlova* species, often located on the long flagellum, but also found on the cell body in some species (Green 1980). Details of scale structure are best observed in stained or shadow cast cell preparations examined under the transmission electron microscope.

One or two chloroplasts, with thylakoids in bands of three but lacking girdle lamella, are present. Pyrenoids may be contained within the chloroplasts or they may bulge out into the cytoplasm. A stigma is associated with the chloroplast in some *Pavlova* species (Green, 1980).

Table 4.1: Classification within the Prymnesiophyceae[1] (Throndsen, 1997).

CLASS: PRYMNESIOPHYCEAE

Order: Isochrysidales Pascher 1910

Family Isochrysidaceae Pascher 1910

- cells with two smooth flagella and a rudimentary haptonema

Genus: *Dicrateria* Parke, 1949

- naked cells (2 spp)

Genus: *Imantonia* Reynolds 1974

- cells with two types of organic scales (1 sp)

Genus: *Isochrysis* Parke, 1949

- cells with one type of organic scale (1 sp)

Order: Coccosphaerales

- with calcified body scales (coccoliths)

(Not studied in the present survey)

Order: Prymnesiales Papenfuss 1955

Family: Prymnesiaceae Conrad 1926

- cells with short or long haptonema, two equal length flaglla

Genus: *Chrysochromulina* Lackey 1939

- cell covering of organic scales (1 - 4 types); homodynamic flagella; short to long and coiling haptonema (> 50 marine spp)

Genus: *Prymnesium* Masart ex Conrad 1926

- cell covering of organic scales (2 or more types); heterodynamic flagella; short haptonema (c. 10 spp)

Genus: *Platychrysis* Geitler 1930

- cell covering of organic scales, typically two types; short haptonema; variable form (4 spp)

Genus: *Corymbellus* Green 1976

- cell covering of organic scales, typically two types; short haptonema; motile cells in colonies (1 sp)

Family Phaeocystaceae Lagerheim 1896

- cells with short haptonema; palmelloid phase dominant (or most conspicuous)

Genus: *Phaeocystis* Lagerheim 1893

- non-motile cells embedded in round or lobed mucilaginous colonies, motile stage *Prymnesium*-like cells (5 spp)

Order Pavlovales Green 1976

- cells with two different flagella, usually one long and one short; long flagellum with knob scales; short haptonema and eyespot may be present

Family Pavlovaceae Green 1976

Genus: *Pavlova* Butcher 1952

- flagella and haptonema inserted subapically (3 marine spp)

Genus: *Diacronema* Prauser 1958

- flattened cells with laterally inserted flagella (1 sp)

[1] A recent modification based on molecular sequencing has been proposed by Edvardsen at al (2000).

4.2 Australian Findings.

Studies from Victorian coastal waters and the East Australian Current identified over 30 prymnesiophyte species, including at least 25 *Chrysochromulina* species (Table 4.2). In addition, Beech (1983) recorded 12 unknown *Chrysochromulina* species, five of which have since been described. The most abundant species reported was *Imantonia rotundata*, with *Chrysochromulina* and *Phaeocystis* species found in both coastal and oceanic waters (Hallegraeff, 1983). In Western Australia, *Prymnesium* blooms are known from brackish waters in the Vasse-Wonnerup estuary and have been related to recurrent fish kills (Hallegraeff, 1992).

In this survey, 40 prymnesiophyte species were reported, including seven new records for Australian waters and two new *Chrysochromulina* species (Table 4.3a,b). Ninteen previously undescribed types of scale were also recorded, but there was insufficient material to describe new species from these scale types.

As there are known toxic harmful algal bloom species, including *Chrysochromulina polylepis* and *Prymnesium patelliferum*, the potential toxicity of species found in this survey was noted from the literature and tested where cultures were available, using the standard *Artemia* nauplii bioassay (as described in Chapter 7).

Table 4.2: Prymnesiophyte species previously recorded from Australian coastal waters (Beech, 1983; Hallegraeff, 1983).

Species	Location	Comments
Chrysochromulina		
C.* aff. *acantha Leadbeater et Manton	Corio Bay, Vic EAC - coastal	
C. adriatica Leadbeater	Hobsons Bay, Vic	
C. alifera Parke et Manton	EAC - coastal	
C. apheles Moestrup et Thomsen	Hobsons Bay, Vic	Listed as *Chrysochromulina* sp. 6 (Beech, 1983)
C. brevifilum Parke et Manton	EAC - oceanic	
C. chiton Parke et Manton	Hobsons Bay, Vic EAC - coastal	
C. cyathophora Thomsen	Corio Bay, Vic.	
C. ephippium Parke et Manton	Hobsons Bay, Vic	
C. ericina Parke et Manton	Hobsons Bay, Vic Corio Bay, Vic	
C. fragaria Eikrem et Edvardsen	Corio Bay, Vic	Listed as *Chrysochromulina* sp. 3 (Beech, 1983)
C. herdlensis Leadbeater	EAC - coastal and oceanic	
C. hirta Manton	Lorne, Vic	
C. leadbeateri Estep, Davis, Hargrave et Sieburth	Hobsons Bay, Vic EAC - coastal and oceanic	Listed as *Chrysochromulina* sp. 7 (Beech, 1983); and *Chrysochromulina* sp. 1 Leadbeater (Hallegraeff, 1983)
C. mantoniae Leadbeater	Hobsons Bay, Vic	
C.* aff. *minor Parke et Manton	EAC - coastal and oceanic	
C. novae-zelandiae Moestrup	EAC - coastal	
C. pachycylindra Manton et Oates	Hobsons Bay, Vic	
C. parkae Green et Leadbeater	Hobsons Bay, Corio Bay, Pt Lonsdale, Vic EAC - coastal and oceanic	
C. polylepis Manton et Parke	Port Phillip Bay	D. Hill (pers. comm.)

Table 4.2 (cont.): Prymnesiophyte species previously recorded from Australian coastal waters (Beech, 1983; Hallegraeff, 1983).

Species	Location	Comments
C. pringsheimii Parke et Manton	Hobsons Bay, Corio Bay EAC - coastal	
C. pyramidosa Thomsen	Airey's Inlet, Vic EAC - coastal	
C. simplex (Estep, Davis, Hargrave et Sieburth) Birkhead et Pienaar	EAC - coastal and oceanic	Listed as *Chrysochromulina* "Plymouth 384"
C. spinifera (Fournier) Pienaar et Norris	Hobsons Bay, Vic	
C.* aff. *vexillifera Manton et Oates	Hobsons Bay, Vic EAC - coastal	Listed as *Chrysochromulina* sp. 1 (Beech, 1983); and described as *C.* aff. *latilepis* (Hallegraeff, 1983)
***Chrysochromulina* sp. "eyelash"**	Hobsons Bay, Vic	Listed as *Chrysochromulina* sp. 12 (Beech, 1983)
Corymbellus aureus Green	EAC - coastal and oceanic	
Imatonia rotunda Reynolds	Ocean Grove, Vic. EAC - coastal and oceanic	
Phaeocystis pouchetii (Hariot) Lagerheim	Hobsons Bay, Corio Bay EAC - coastal and oceanic	
Phaeocystis scrobiculata Moestrup	Hobsons Bay EAC - coastal and oceanic	
Platychrysis pienaarii Gayral et Fresenel	Tarranyurk, Vic	
Prymnesium patelliferum Green, Hibberd et Pienaar	Shallow Inlet, Corio Bay	
***Prymnesium* sp**	Vasse-Wonnerrup estuary, WA	(Hallegraeff, 1992)
***Pavlova* spp**	EAC - coastal and oceanic	

EAC = East Australian Current

Table 4.3a: *Chrysochromulina* species found in southern Tasmanian waters and their overall distribution.

Species	Present Findings (Site Code)	Growth in Enrichment Culture	New Record for Australia	Recorded Distribution
Chrysochromulina				
C. acantha Leadbeater et Manton	DER	GSe	Yes	Temperate, coastal (northern hemisphere)
C. adriatica Leadbeater	DER, STB, PCL, EN	GSe,GSe/2	-	Temperate, coastal (only 2 reports)
C.* aff. *ahrengotii [1] Jensen et Moestrup	PCL, DP	-	Yes	Temperate, coastal (northern hemisphere)
C. alifera Parke et Manton	DP	-	-	Temperate, coastal
C. apheles Moestrup et Thomsen	DER	GSe	-	Subpolar to tropical, coastal
C.* aff. *brachycylindra [4] Hällfors et Thomsen	PCL	-	Yes	Subpolar to tropical, coastal (only 2 reports)
C. brevifilum [3] Parke et Manton	DER, DP, DB, FP, LSP, CB	GSe/10, ML	-	Temperate, coastal
C.* aff. *camella Leadbeater et Manton	PB	-	Yes	Temperate, coastal
C. chiton Parke et Manton	PCL	-	-	Temperate, coastal, (1 oceanic record)
C. cyathophora Thomsen	SPT	-	-	Temperate to subpolar, coastal
C. ephippium Parke et Manton	FP	-	-	Temperate to subpolar, coastal and oceanic
C. ericina [3] Parke et Manton	DER, PCL, OB, HMB	GSe, ML, K/2	-	Temperate, coastal
C. fragaria Eikrem et Edvardsen	DER, DP, OCP, DB	-	-	Temperate, coastal (only 2 reports)
C. hirta [3] Manton	DER, STB, DP, SP, OCP, FP, DB, SPT, PCL, RB, PB, HMB	GSe, GSe/2, ML, K, f-Si/100	-	Polar to subtropical, coastal (1 oceanic record)
C. leadbeateri [2, 3] Estep, Davis, Hargrave et Sieburth	PCL, SPT	-	-	Subpolar to subtropical, coastal and oceanic
C. mactra Manton	PB	-	Yes	Temperate, coastal
C. mantoniae Leadbeater	PH	-	-	Temperate, coastal
C. minor Parke et Manton	PCL, CB	-	-	Temperate, coastal

Table 4.3a (cont.): *Chrysochromulina* species found in southern Tasmanian waters and their overall distribution.

Species	Present Findings (Site Code)	Growth in Enrichment Culture	New Record for Australia	Recorded Distribution
C. novae-zelandiae Moestrup	DP, FP	-	-	Temperate, coastal (southern hemisphere)
C. pachycylindra Manton et Oates	DER, DP, PCL, EN, HMB, CB, STB	GSe, GSe/10, ML	-	Temperate to subtropical, coastal and oceanic
C. parkae Green et Leadbeater	DER, DP, SPT, PCL, EN, HMB, PB	GSe, K/10	-	Temperate, coastal (1 oceanic record)
C. **aff.** *polylepsis* [2] Manton et Parke	HMB	-	-	Temperate, coastal
C. pringsheimii Parke et Manton	DER, CP, PCL, OCP, EN, HMB	GSe/10, ML	-	Temperate, coastal
C. pyramidosa Thomsen	SPT, HMB	GSe, ML	-	Temperate, coastal
C. **aff.** *scutellum* [1] Eikrem et Moestrup	DER, PCL, SPT	GSe	Yes	Temperate, coastal
C. simplex (Estep, Davis, Hargrave et Sieburth) Birkhead et Pienaar	DER, DB	GSe, GSe/2	-	Temperate, coastal and oceanic
C. **cf.** *simplex*	DER, DP	-	-	
C. spinifera [3] (Fournier) Pienaar et Norris	DER, OCP, FP, DB, SPT, PCL, HMB, PCL, LSP	GSe, GSe/10	-	Temperate, coastal
C. **aff.** *vexillifera* Manton et Oates	PCL	-	-	Temperate, coastal and oceanic
Chrysochromulina "eyelash"	DER, CP, DP, PH, MI	GSe, GSe/2, GSe/10, ML	-	Temperate to tropical, coastal
Chrysochromulina **spp (19)**				

[1] First report since orginal record and first report for southern hemisphere

[2] Potentially toxic species

[3] Implicated in harmful algal blooms

[4] First report for southern hemisphere

Table 4.3b (cont.): Other Prymnesiophyte species found in southern Tasmanian waters and their overall distribution.

Species	Present Findings (Site Code)	Growth in Enrichment Culture	New Record for Australia	Recorded Distribution
Corymbellus aureus Green	CP, DP, HMB	-	-	Temperate, coastal and oceanic
Imantonia rotunda Reynolds	DER, CP, DP, FP, OCP, SPT, PH, RB, PCL, PB, HMB, CB	GSe, GSe/2, GSe/10, K/2, ML	-	Polar to subtropical, coastal and oceanic
Phaeocystis globosa [3] Scherffel	DER, CP, DP, OCP, FP, PH, SPT, PCL, RB, HMB, LSP, PB, MI	GSe	-	Temperate to subtropical, coastal and oceanic
Phaeocystis scrobiculata Moestrup	DER, PCL, HMB	-	-	Temperate to tropical, coastal and oceanic
Prymnesium nemamethecum Pienaar et Birkhead	DP	-	-	Temperate, coastal
Prymnesium patelliferum [2, 3] Green, Hibberd et Pienaar	LSP, PCL	-	-	Temperate, coastal
Pavlova pinguis Green	PCL	GSe	Yes	Temperate, coastal and oceanic
Pavlova sp	PCL, SPT, CB	ML	-	

[1] First report since orginal record and first report for southern hemisphere

[2] Potentially toxic species

[3] Implicated in harmful algal blooms

[4] First report for southern hemisphere

4.3 Species Descriptions

***Chrysochromulina acantha* Leadbeater et Manton** **Figs. 4.1 - 4.5**

Micrographs: Leadbeater and Manton, 1971; Figs. 9 - 12.

Present Findings.
Whole cells and scales were identified from GSe enrichment cultures derived from Derwent River samples. Individual cells were isolated and a unialgal culture (CS-480) was successfully maintained in the CSIRO Collection of Living Microalgae for three years.

Chrysochromulina acantha is reported here as a new record for Australian waters.

Description.
In culture, cells measured approximately 10 µm, and had two equal flagella and a long coiling haptonema, 40 µm in length when fully extended.

Two types of scales, oval plate scales and spine scales, were seen (Fig. 4.1). Plate scales ranged in size from 0.6 - 0.8 x 0.5 - 0.8 µm ($\bar{x}$=0.7 x 0.5 µm; n=7). Each scale was patterned with 36 - 38 evenly-spaced ridges, which radiated from the scale centre to a thickened margin and were superimposed on 8 - 9 concentric rings (Fig. 4.2).

Spine scales were generallly circular, ranging from 0.4 - 0.6 µm diameter ($\bar{x}$=0.5 µm; n=17). Each had a central spine, 0.5 - 0.6 µm in height ($\bar{x}$=0.58 µm; n=17), attached to a base plate by four decurrent struts extending to a thickened margin. Fewer radiating ridges and concentric rings were found patterning base plate surfaces in comparison to plate scales; there were 26 - 28 radiating ridges superimposed on 7 - 8 concentric rings. The same pattern was found on both proximal and distal surfaces (Figs. 4.3, 4.4).

These scales closely matched the type description (Leadbeater and Manton, 1971). After examining both wild and cultured cells, Leadbeater and Manton commented on the consistent scale size and patterning, in contrast to other *Chrysochromulina* species, for example, *C. ericina* and *C. chiton,* which show considerable variation in scale dimension and structure.

Given the number of differences in scale structure and size, scales described as *C.* aff. *acantha* by Beech (1983; Plate 2.1 A- B), Hallegraeff (1983; Fig. 5), and Rhodes and Burke (1996; Fig. 2), were most likely from other *Chrysochromulina* species.

Although all these described spine scales had a central spine attached to a base plate by four decurrent ridges, the pattern on the base plate usually included a higher number of radiating ridges (Table 4.4). Scales from Sydney coastal waters and New Zealand were almost twice the size of scales from the type species, while scales from Corio Bay, Victoria, had slightly shorter spines, decurrent ridges that did not always reach scale edges, and raised peripheral rims (Beech, 1983; Plate 2.1A).

Plate scales also varied from the type species description (Table 4.4). The plate scales described by Hallegraeff (1983; Fig. 5a, c) had different patterns on proximal and distal surfaces as well as almost twice the number of radiating ridges. In addition, scales shown in Fig. 5b (Hallegraeff, 1983) had patterned margins. Plate scales described by Beech (1983; Plate 2.1B) had approximately 50 radiating ridges and a "thin inflexed rim".

In this survey, spine scales similar to those of *C. acantha* were found in a Pipeclay Lagoon sample (Fig. 4.5). These had a similar spine height but a larger base plate diameter and numerous concentric rings (Table 4.4).

Distribution.

C. acantha was originally described from England (Leadbeater and Manton, 1971) and later from Norway (Leadbeater, 1972a; Edvardsen and Paasche, 1992), Denmark (Manton and Leadbeater, 1974; Dahl et al, 1998), Yugoslavia (Leadbeater, 1974) and Greenland (Thomsen, 1982).

Toxicity.

C. acantha has been reported as non-toxic to *Artemia* nauplii (Edvardsen and Paasche, 1992). However, in the present study, *Artemia* nauplii fed stationary phase cultures of *C. acantha* markedly slowed their swimming activity (see Chapter 7).

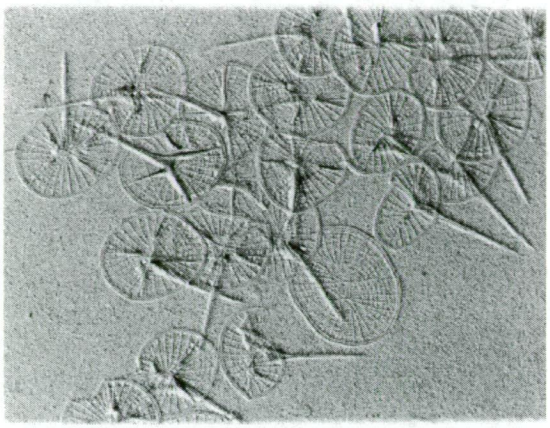


Fig. 4.1: *C. acantha* plate and spine scales; from a Derwent enrichment culture

(Micrograph no: 4782)

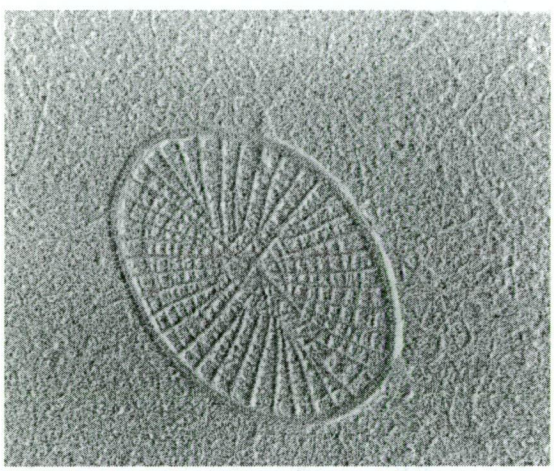


Fig. 4.2: *C. acantha* plate scale (0.7 x 0.5 μm); from a Derwent enrichment

(Micrograph no: 4791)

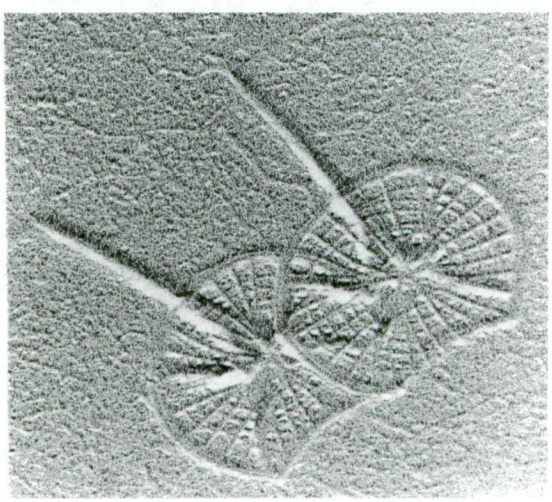


Fig. 4.3: *C. acantha* spine scales (0.5 μm diameter) - proximal view; from a Derwent enrichment culture

(Micrograph no: 4792)

Fig. 4.4: *C. acantha* spine scales (0.5 μm diameter) - distal view; from a Derwent enrichment culture

(Micrograph no: 5324)

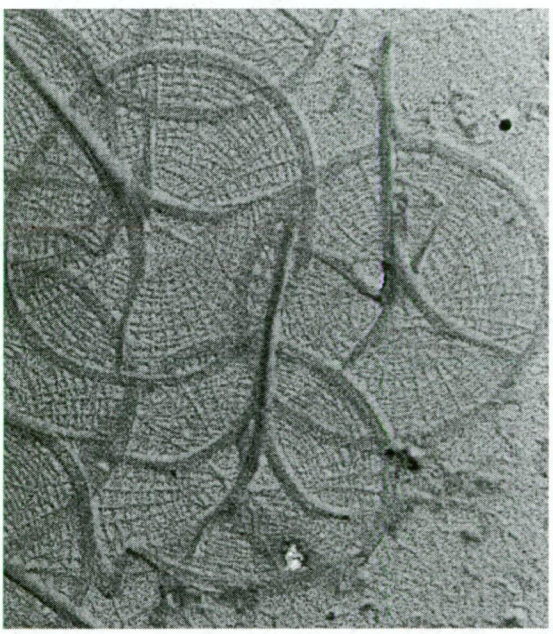


Fig. 4.5: *C.* aff. *acantha* spine scales (0.7 μm diameter); from Pipeclay Lagoon

(Micrograph no: 5433)

Table 4.4: Scales of *Chrysochromulina acantha* compared with *Chrysochromulina* aff. *acantha*.

SOURCE	PLATE SCALES				SPINE SCALES				
	Length (μm)	*Width (μm)*	*No. of Ridges*	*No. of Rings*	*Length (μm)*	*Width (μm)*	*Height (μm)*	*No. of Ridges*	*No. of Rings*
Chrysochromulina acantha									
UK *(type)*	0.6	0.5	36	7 - 9	0.4 - 0.5	0.4 - 0.5	c. 0.5	28	7 - 9
(Leadbeater & Manton, 1971)	($\bar{x}$ =0.62)	($\bar{x}$ =0.52)			($\bar{x}$ =0.45)	($\bar{x}$ =0.45)			
Tasmania, Australia	0.6 - 0.8	0.5 - 0.8	36 - 38	8 - 9	0.4 - 0.6	0.4 - 0.6	0.5 - 0.6	26 - 28	7 - 9
	($\bar{x}$ =0.7; n=7)	($\bar{x}$ =0.5; n=7)			($\bar{x}$ =0.5; n=17)	($\bar{x}$ =0.5; n=17)	($\bar{x}$ =0.6; n=16)		
Chrysochromulina* aff. *acantha									
New South Wales, Australia (Hallegraeff, 1983)	0.6 - 0.8	0.5 - 0.7	c. 70	ND	1.0	0.8	1.1	c. 40	ND
Victoria, Australia (Beech, 1983)	0.5 (n=1)	0.4 (n=1)	c. 50	ND	0.4 - 0.5 ($\bar{x}$ =0.42; n=2)	0.4 ($\bar{x}$ =0.4; n=2)	0.3 (n=1)	c. 60	ND
New Zealand (Rhodes & Burke, 1996)	-	-	-	-	1.1 (n=1)	1.0 (n=1)	0.9 (n=1)	c. 30	ND
Tasmania, Australia	-	-	-	-	0.7 (n=2)	0.7 (n=2)	0.6 (n=2)	36	>20

ND = Not determined

***Chrysochromulina adriatica* Leadbeater** **Figs. 4.6 - 4.8**

Micrographs: Leadbeater, 1974; Plate 6A - C.
Beech, 1983; Plate 2.1 C - D.

Present Findings.
Scales were found in a sample from Eaglehawk Neck, and both scales and whole cells were seen in Derwent River and Pipeclay Lagoon samples.

Description.
Whole cells were 3.0 - 3.5 x 2.5 - 3.0 μm (n=2). They had two equal flagella, 7 - 9 μm in length ($\bar{x}$=8 μm; n=4), and a slightly shorter haptonema, 4 - 6 μm ($\bar{x}$=5; n=2) (Fig. 4.6). Cells were smaller than the type species, but flagellar and haptonemal lengths were similar (Table 4.5). Cell dimensions given in the type description were only estimated by Leadbeater (1974) who measured the length and width of the scale case and made allowance for protoplast shrinkage during sample preparation. However, the cell size measured directly from a micrograph of the type species was 3.3 x 2.6 μm (Leadbeater, 1974; Plate 6A), which was in the same size range as the Tasmanian material.

There were two types of plate scales, both with similar patterning of radiating ridges. The first scale type was circular, 0.6 - 0.8 μm diameter ($\bar{x}$=0.7; n=10), and had a distinct raised rim, 0.06 - 0.08 μm wide ($\bar{x}$=0.07; n=3). One surface was patterned with 30 - 34 slightly curved radiating ridges, arranged in quadrants and extending to the scale rim, superimposed on concentric fibrils (Fig. 4.7); the opposite surface was patterned with irregularly arranged fibrils (Fig. 4.8).

The second scale type was oval, 0.6 - 0.8 x 0.4 - 0.5 μm ($\bar{x}$ =0.7 x 0.5 μm; n=15) and patterned with 28 straight radiating ridges, not arranged in quadrants but evenly-spaced and superimposed on spirally arranged fibrils. A narrow peripheral band was composed of faint concentric fibrils (Fig. 4.8). This scale type could be easily confused with the plate scales of *C. acantha* or *C. pyramidosa,* given the similarities in size and surface patterning.

Both scale types were considerably smaller than those of the type species, but were similar in size to scales described from Australian waters (Table 4.5).

Scale structure closely matched that given for the type species and for the Australian material (Leadbeater, 1974; Beech, 1983), with additional information from the Tasmanian material including rim width, number of radiating ridges, and different surface patterning on both sides of the rimmed scales.

Distribution.

C. adriatica was originally described from Yugoslavia (Leadbeater, 1974), and has since been reported only from south-east Australia (Beech, 1983).

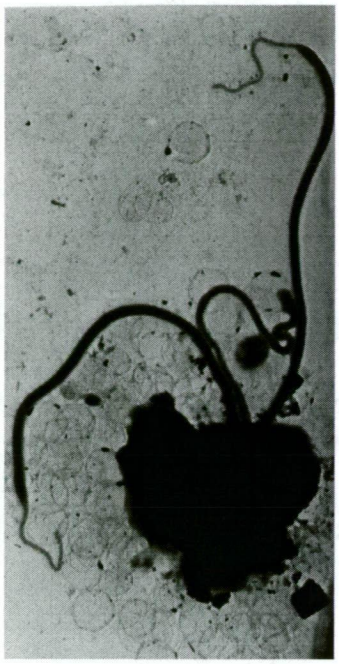


Fig. 4.6: *C. adriatica* cell (c. 3 μm), showing two flagella and haptonema; from the Derwent River

(Micrograph no: 5334)

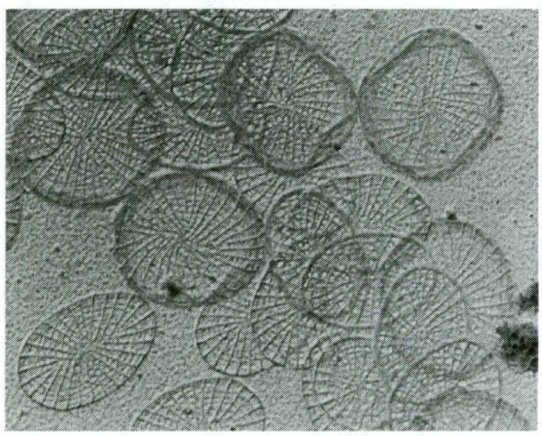


Fig. 4.7: *C. adriatica* rimmed scales (0.7 μm) and oval scales (0.7 x 0.5 μm); from Eaglehawk Neck

(Micrograph no: 5414)

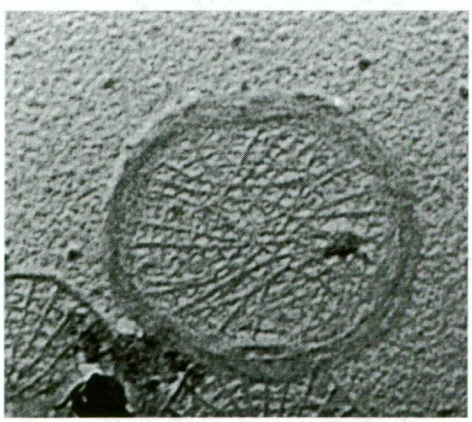


Fig. 4.8: *C. adriatica* rimmed scale (0.7 μm), showing irregular patterning of fibrils; from Eaglehawk Neck

(Micrograph no: 5414)

Table 4.5: *Chrysochromulina adriatica* from different locations.

SOURCE	CELL SIZE	FLAGELLA	HAPTONEMA	RIMMED PLATE SCALES			OVAL PLATE SCALES	
	Dimensions (*µm*)	*Length* (*µm*)	*Length* (*µm*)	*Dimensions* (*µm*)	*Rim Width* (*µm*)	*No. of Ridges*	*Dimensions* (*µm*)	*No. of Ridges*
Yugoslavia (Leadbeater, 1974)	5 - 8 x 6 - 9 (3.3 x 2.6)*	8 - 10	6 - 10	1.8 - 2.5	0.1	c. 30	1.8 - 2.5 x 1.2 - 1.5	30 - 32
Australia (Beech, 1983)	3.5 x 3.0 (n=1)	c. 6	-	0.75 (n=2)	0.08	c. 32	0.65 x 0.4 (n=2)	26
Tasmania, Australia	3.0 - 3.5 x 2.5 - 3.0 (n=2)	7 - 9 ($\bar{x}$ =5; n=4)	4 - 6	0.6 - 0.8 ($\bar{x}$ =0.7; n=10)	0.06 - 0.08 ($\bar{x}$ =0.07; n=10)	30 - 34	0.6 - 0.8 x 0.4 - 0.5 ($\bar{x}$ =0.7 x 0.5; n=15)	28

* Direct measurement from published micrograph

***Chrysochromulina* aff. *ahrengotii* Jensen et Moestrup** **Figs. 4.9 - 4.10**

Micrographs: Jensen and Moestrup, 1999; Figs. 4 - 12.

Present Findings.
Whole cells were observed in a GSe/10 (+GeO_2) enrichment culture derived from a Dru Point sample. Spine and plate scales were found in a Pipeclay Lagoon sample.

This is a new record for Australian waters, and the first report from the southern hemisphere. It is also the first report since the orginal species description (Jensen and Moestrup, 1999).

Description.
Cells were approximately 4 µm, and had two equal flagella and a long coiling haptonema, at least 50 µm when extended. Both plate scales and spine scales were observed (Fig. 4.9).

Plate scales were oval, with a size range of 0.5 - 0.6 x 0.4 - 0.5 µm ($\bar{x}$=0.57 x 0.44 µm; n=10), and it was difficult to distinguish their surface patterning. Spine scales were circular to oval, 0.29 x 0.24 - 0.29 µm ($\bar{x}$=0.29 x 0.25 µm; n=5), with a central spine, 0.21 - 0.30 µm long ($\bar{x}$=0.26; n=4), attached to the base plate by four decurrent struts extending to the scale margin. The base plate was patterned with c. 36 radiating ridges (arranged in quadrants) and faint concentric fibrils, and had a narrow marginal thickening (Fig. 4.10). These spine scales differed from the Danish type material (Jensen and Moestrup, 1999) in having more radiating ridges on the base plate of the spine scale, and a spine length which was sometimes less than the scale diameter. Both spine and plate scales were within the appropriate size ranges when compared with scales of the type species (Table 4.6).

Spine scales may be confused with those of *C. ephippium* or *C. scutellum* given their similar size and structure (Table 4.6). *C. ephippium* scales have a spine length which is usually greater than the scale diameter, and an upright outer rim (Parke et al, 1956; Manton and Leadbeater, 1974). In *C. scutellum* spine scales lacking an upright rim, spine length is only slightly greater than the scale radius, and there is a more distinct pattern of concentric fibrils on the base plate surface (Eikrem and Moestrup, 1998; Fig. 14).

Distribution.

C. ahrengotii was originally described as a fairly common species from Danish waters, found at salinities between 8 and 30 psu and at temperatures ranging from 10 to 23°C (Jensen and Moestrup, 1999).

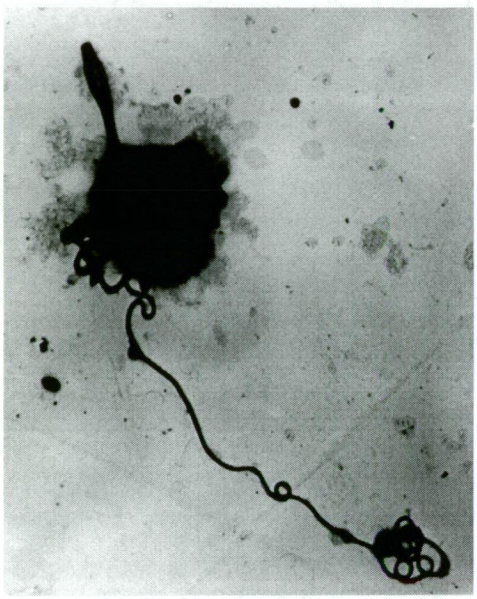


Fig. 4.9: *C.* aff. *ahrengotii* cell (c. 4 μm), showing long haptonema; from a Dru Point enrichment culture

(Micrograph no: 5240)

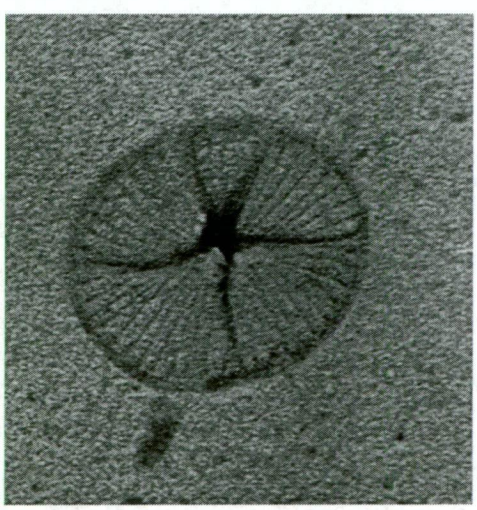


Fig. 4.10: *C.* aff. *ahrengotii* spine scale (0.25 μm) - proximal view; from Pipeclay Lagoon

(Micrograph no: 5216)

Table 4.6: Scales of *Chrysochromulina ahrengotii* compared with *C.* aff. *ahrengotii, C. ephippium* and *C. scutellum.*

SOURCE	PLATE SCALES				SPINE SCALES			
	Length (μm)	*Width (μm)*	*No. of Ridges*	*Inflexed Rim*	*Diameter (μm)*	*Height (μm)*	*No. of Ridges*	*Upright Rim*
Chrysochromulina ahrengotii								
Denmark *(type)*	0.28 - 0.69	0.25 - 0.46	c. 40	Yes	0.28 - 0.48	0.20 - 0.43	c. 24	No
(Jensen & Moestrup, 1999)	(0.52 - 0.61*; n=166)	(0.38 - 0.45*; n=164)			(0.32 - 0.40*; n=143)	(0.23 - 0.43*; n=123)		
Chrysochromulina* aff. *ahrengotii								
Tasmania, Australia	0.5 - 0.6	0.4 - 0.5	ND	ND	0.24 - 0.29	0.21 - 0.30	c. 36	No
	($\bar{x}$ =0.57; n=10)	($\bar{x}$ =0.44; n=10)			($\bar{x}$ =0.25; n=5)	($\bar{x}$ =0.26; n=4)		
Chrysochromulina ephippium								
UK *(type)*	0.5 - 0.7	0.5 - 0.7	c. 65	Yes	0.3 - 0.6	≥ scale diameter	c. 40	Yes
(Parke et al, 1956)								
Denmark	0.5 - 0.6	0.5 - 0.6	c. 65	Yes	0.3 - 0.6	>>scale diameter	c. 40	Yes
(Manton & Leadbeater, 1974)								
Chrysochromulina scutellum								
Norway, Denmark *(type)*	0.6 - 0.7	0.5 - 0.6	c. 56	Yes	**(1)** 0.3 - 0.45	≥ spine radius	c. 28 - 32	No
(Eikrem & Moestrup, 1998)					**(2)** 0.25 - 0.35	≤ spine radius	c. 24	Yes

ND = Not determined

* Most common size range of scales

***Chrysochromulina alifera* Parke et Manton** **Figs. 4.11 - 4.14**

Micrographs: Parke et al, 1956; Figs. 74 - 76.

Present Findings.
Scales were found in water samples from Dru Point.

Description.
The two scale types of *Chrysochromulina alifera* are shown in Fig. 4.13. Both plate and spine scales were circular to oval and were patterned on the distal surface with c. 40 evenly-spaced radiating ridges overlying faint concentric fibrils.

Plate scales had a size range of 0.38 - 0.40 x 0.26 - 0.29 µm ($\bar{x}$=0.38 x 0.29; n=9). On the proximal surface, a pattern of numerous faint concentric fibrils was observed, as well as a broad-banded outer rim (Fig. 4.11). The rim appeared to be inflexed when viewed from the distal side (Figs. 4.12, 4.13).

Spine scales were 0.32 - 0.33 µm in size ($\bar{x}$=0.33 µm; n=2), with a central spine of 0.23 - 0.29 µm ($\bar{x}$=0.26; n=2), slightly shorter than the scale diameter. The spine appeared to be supported by short decurrent struts which did not extend to the scale's upright rim. There did not seem to be any differences in patterning on the proximal and distal surfaces (Figs. 4.12, 4.13).

These scale types closely matched those of the type species, as did the material described by Moestrup (1979) from New Zealand (Table 4.7). *C. alifera* scales may be difficult to differentiate from those of *C. epihippium,* or the more recently described species, *C. ahrengotii* and *C. scutellum.* For example, a spine scale of *C. alifera* recorded from Australian coastal waters (Hallegraeff, 1983; Fig. 8) was more likely to belong to *C. ephippium,* as the spine length was greater than the scale diameter and the spine struts extended to the scale rim (Parke et al, 1956; Manton and Leadbeater, 1974). *C. ahrengotii* plate scales are larger in size than those of *C. alifera,* and spine scales lack an upright rim and have fewer radiating ridges on the scale surface (Jensen and Moestrup, 1999). *C. scutellum* has smaller plate scales, and spine scales without an upright rim have fewer radiating ridges (Eikrem and Moestrup, 1998).

A field of scales similar to those of *C. alifera* was observed in one of the Dru Point water samples (Fig. 4.14). The obvious difference was two distinct central perforations present on the plate scales. Scale dimensions are compared in Table 4.7.

Distribution.

C. alifera has been reported from both the northern and southern hemispheres, namely from: UK, Norway, Denmark, Algeria, Yugoslavia, New Zealand and Australia (Moestrup, 1979, and references therein; Hallegraeff, 1983). Of these records, micrographs were published only for the UK type (Parke et al, 1956) and the Australian material (Hallegraeff, 1983), and it is possible that other cells or scales described were confused with different *Chrysochromulina* species.

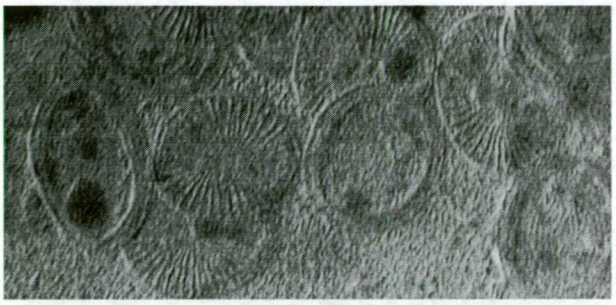


Fig. 4.11: *C. alifera* plate scales (c. 0.3 μm) - proximal and distal views; from Dru Point

(Micrograph no: 4903)

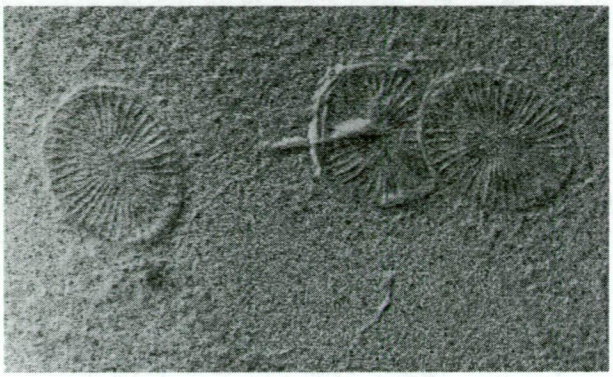


Fig. 4.12: *C. alifera* spine scale (c. 0.3 μm) - proximal view; plate scales (0.4 x 0.3 μm) - distal view; from Dru Point

(Micrograph no: 4902)

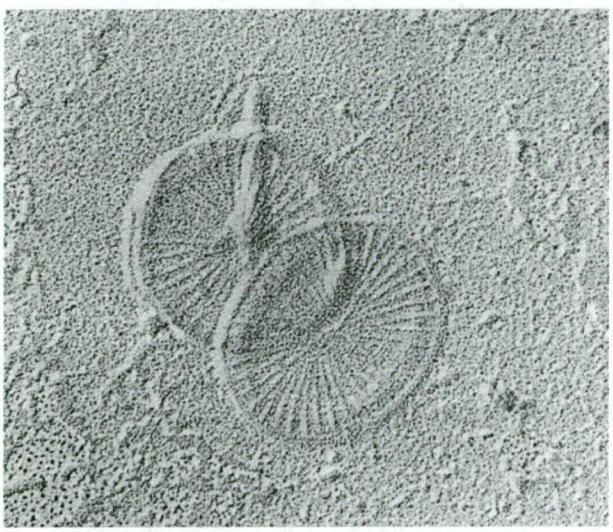


Fig. 4.13: *C. alifera* spine scale (c. 0.3 μm) and plate scale (0.4 x 0.3 μm) - distal view; from Dru Point

(Micrograph no: 4821)

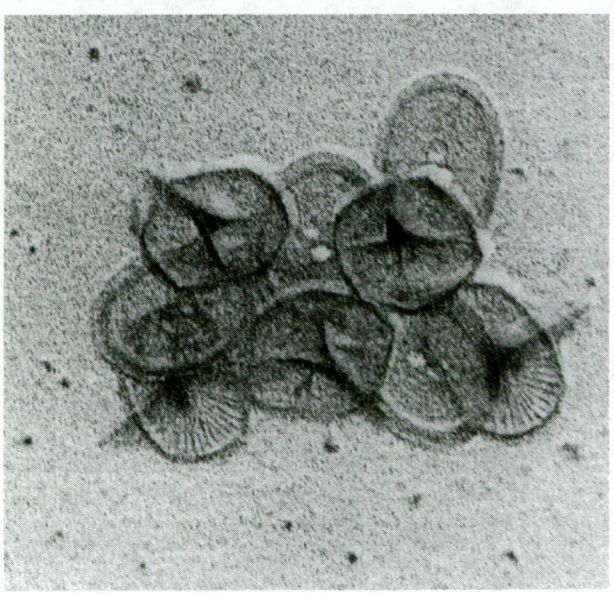


Fig. 4.14: *C.* cf. *alifera* spine and plate scales (c. 0.5 μm), with central perforations seen on plate scales; from Dru Point

(Micrograph no: 5173)

Table 4.7: Scales of *Chrysochromulina alifera* compared with *C.* cf. *alifera*.

SOURCE	PLATE SCALES			SPINE SCALES			
	Dimensions (µm)	*No. of Ridges*	*Inflexed Rim*	*Dimensions (µm)*	*Height (µm)*	*No. of Ridges*	*Upright Rim*
Chrysochromulina alifera							
UK *(type)* (Parke et al, 1956)	0.28 - 0.45	Numerous	Yes	0.25 - 0.45	< scale diameter	c. 38 - 42	Yes
New Zealand (Moestrup, 1979)	0.23 - 0.41	ND	ND	0.26 - 0.41	ND	ND	ND
Tasmania, Australia	0.38 - 0.40 x 0.26 - 0.29 ($\bar{x}$ =0.38 x 0.29; n=9)	c. 40	Yes	0.32 - 0.33 ($\bar{x}$ =0.325; n=2)	0.23 - 0.29 ($\bar{x}$ =0.26; n=2)	c. 40	Yes
Chrysochromulina* cf. *alifera							
Tasmania, Australia	0.59 - 0.65 x 0.44 - 0.50 ($\bar{x}$ =0.60 x 0.47; n=3)	ND	Yes	0.44 - 0.56 x 0.39 - 0.44 ($\bar{x}$ =0.52 x 0.40; n=5)	0.33 - 0.38 ($\bar{x}$ =0.35; n=4)	c. 40	Yes

ND = Not determined

***Chrysochromulina apheles* Moestrup et Thomsen** **Figs. 4.15 - 4.16**

Micrographs: Moestrup and Thomsen, 1986; Fig. 17.
Thomsen, 1986; Fig. 18.

Present Findings.
Whole cells and scales were found in GSe and GSe/2 enrichment cultures derived from Derwent River samples. Individual cells were isolated and a unialgal culture (CS-481) is currently maintained in the CSIRO Living Collection of Microalgae (in GSe medium at 15°C, under standard growth conditions).

Description.
In culture, cells were approximately 4 μm. Cells had two unequal flagella (8 - 11 μm) and a long coiling haptonema, c. 40 μm when fully extended.

Two types of scales were observed (Figs. 4.15, 4.16). The larger scale type, 0.19 - 0.24 μm ($\bar{x}$=0.21 μm, n=34), had an upright rim and a slightly angular appearance. In the scale centre, a cross separating four perforations was surrounded by a distinct concentric ring. Between this central area and the scale periphery, there were 16 - 19 evenly-spaced radiating ridges overlying concentric rings. The smaller scale type was circular with a 0.15 - 0.19 μm diameter ($\bar{x}$=0.17 μm; n=34), and an upright rim. It had similar surface patterning, but with slightly fewer radiating ridges (15 - 16) and concentric rings, and was less numerous than the larger scales.

These observations closely agreed with the type description given by Moestrup and Thomsen (1986).

A species with some similarities to *C. apheles* was described from Australian waters by Beech (1983). Cells were approximately 4 μm with two flagella (8 - 12 μm), and a long coiling haptonema. Only one scale type was recorded, but this had a structure similar to that of the smaller *C. apheles* scale, being circular and rimless with a central cross and radiating ridges overlying concentric rings. (Beech, 1983; Plate 2.12B). It was larger than the typical *C. apheles* scale, having a 0.22 - 0.29 μm diameter ($\bar{x}$=0.24 μm, n=8). Scale patterning included 19 - 21 radiating ridges, which appeared to be arranged in quadrants rather than evenly-spaced, and there were only two central perforations.

Moestrup and Thomsen (1986) noted that some *C. apheles* scales contained two or three central perforations rather than four. For example, *C. apheles* (PCC-385) from the Plymouth Culture Collection had numerous scales with only two central holes.

C. apheles scales from different locations are compared in Table 4.8.

Distribution.

Scales similar to those of *C. apheles* have been observed in coastal water samples from Denmark, Finland, England, Thailand, Australia and New Zealand, and have been found at temperatures ranging from 8 - 28 °C and salinities from 5.8 - 35 psu (Moestrup and Thomsen, 1986).

Toxicity.

C. apheles has been reported as non-toxic to *Artemia* nauplii (Simonsen and Moestrup, 1997), which agreed with findings in this study (see Chapter 7).

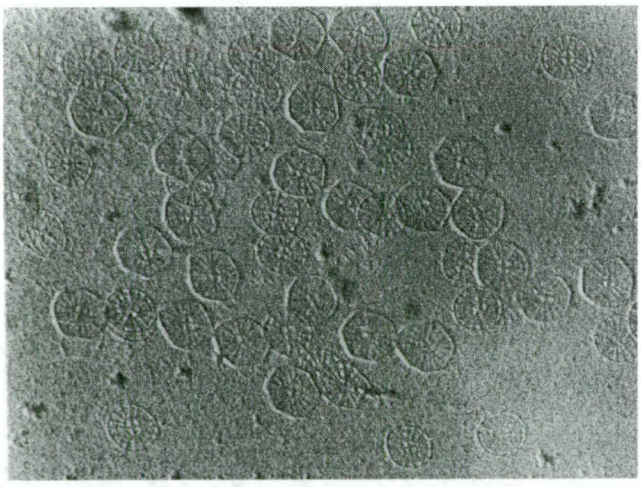


Fig. 4.15: *C. apheles* field of scales; from a Derwent enrichment culture

(Micrograph no: 4836)

Fig. 4.16: *C. apheles* angular and circular scales (c. 0.2 μm); from the Derwent River

(Micrograph no: 4673)

Table 4.8: Scales of *Chrysochromulina apheles* from different locations.

SOURCE	LARGE SCALES			SMALL SCALES		
	Diameter (μm)	*No. of Ridges*	*No. of Perforations*	*Diameter (μm)*	*No. of Ridges*	*No. of Perforations*
Chrysochromulina apheles						
Denmark *(type)* (Moestrup & Thomsen, 1986)	0.20 - 0.25	19 - 21	2 - 4	0.16 - 0.21	15 - 17	4
New Zealand (Moestrup & Thomsen, 1986)	ND	ND	ND	0.26 - 0.27	ND	ND
Thailand (Moestrup & Thomsen, 1986)	ND	ND	ND	0.23 - 26	ND	ND
Tasmania, Australia	0.19 - 0.24 ($\bar{x}$ =0.21; n=34)	16 - 19	4	0.15 - 0.19 ($\bar{x}$ =0.17; n=34)	15 - 16	4
Chrysochromulina* aff. *apheles						
Victoria, Australia (Beech, 1983)	-	-	-	0.22 - 0.29 ($\bar{x}$ =0.24; n=8)	19 - 22	2

ND = Not determined

***Chrysochromulina* aff. *brachycylindra* Hällfors et Thomsen** **Fig. 4.17**

Micrographs: Hällfors and Thomsen, 1985; Figs. 10 - 12.

Present Findings.
Scales were found in a sample from Pipeclay Lagoon.

This is a new record for Australian waters, and the first report from the southern hemisphere.

Description.
Two scale types were observed (Fig. 4.17). One scale type was oval, with dimensions of 0.70 - 0.72 x 0.55 - 0.60 µm ($\bar{x}$=0.71 x 0.58 µm; n=2), and a distinct patternless upright rim, 0.26 - 0.28 µm high (n=2). The second scale type was circular to oval, and slightly smaller, 0.58 - 0.67 x 0.53 - 0.58 µm ($\bar{x}$=0.61 x 0.56 µm; n=5). It had a pattern of c. 80 radiating ridges in quadrants on one surface, and numerous faint concentric fibrils and a broad peripheral band, c. 0.05 µm wide, on the opposite surface.

These scales were similar to those of *C. brachycylindra* (Table 4.9). However, plate scales of *C. brachycylindra* have a infra-marginal rim, about 0.1 µm inside the the scale edge (Hällfors and Thomsen, 1985; Figs. 10 - 11). Rimmed scales have a central oblong thickening and a concentrically striated rim (Hällfors and Thomsen, 1985; Figs. 12 and 14). Neither of these features were observed in the Tasmanian material.

Distribution.
C. brachycylindra was originally described from Finland and Thailand, at temperatures ranging from 14 - 28 °C and salinities from 6 - 34 psu (Hällfors and Thomsen, 1985). Since its original description, there has been only one other report of this species which was from the northern Baltic Sea, off the east coast of Sweden (Hadju et al, 1996).

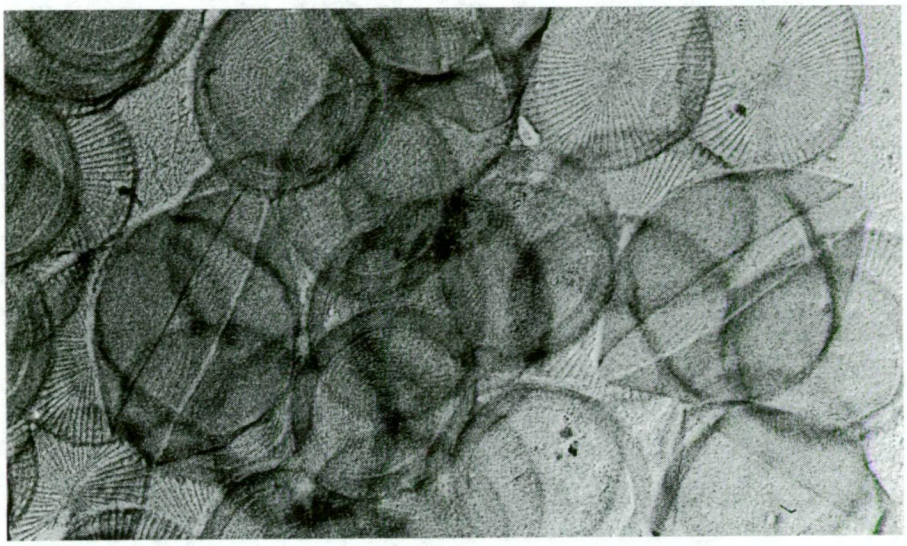


Fig. 4.17: *C.* aff. *brachycylindra* scales: oval plate scales (0.7 x 0.6 μm) with a distinct rim, and circular scales (c. 0.6 μm) with a broad peripheral band; from Pipeclay Lagoon

(Micrograph no: 4836)

Table 4.9: Scales of *Chrysochromulina brachycylindra* compared with *C.* aff. *brachycylindra.*

SOURCE	PLATE SCALES WITH RIM			PLATE SCALES	
	Dimensions (μm)	*Rim Height*	*No. of Ridges*	*Dimensions (μm)*	*No. of Ridges*
Chrysochromulina brachycylindra					
Finland *(type)* (Hällfors & Thomsen, 1985)	1.0 - 1.6 x 0.6 - 1.2	0.4 - 0.8	86 - 144	0.9 - 1.5 x 0.7 - 1.2	80 - 128
Thailand (Hällfors & Thomsen, 1985)	0.7 - 1.0 x 0.5 - 0.8	0.2	ND	0.8 x 0.6 - 0.7	ND
Chrysochromulina* aff. *brachycylindra					
Tasmania, Australia	0.70 - 0.72 x 0.55 - 0.60 ($\bar{x}$ =0.71 x 0.58; n=2)	0.26 - 0.28 ($\bar{x}$ =0.27; n=2)	ND	0.58 - 0.67 x 0.53 - 0.58 ($\bar{x}$ =0.61 x 0.56; n=5)	c. 80

ND = Not determined

***Chrysochromulina brevifilum* Parke et Manton** **Fig. 4.18**

Micrographs: Parke et al, 1955; Figs. 80 - 81.
Birkhead and Pienaar, 1994; Figs. 10 - 18.

Present Findings.
Scales were found in Derwent River samples, and both scales and whole cells were seen in Deep Bay and Fleurty Point samples.

Chrysochromulina brevifilum grew in GSe/10 (+GeO_2) enrichment cultures derived from a Dru Point sample. Scales were found in ML enrichment cultures from Little Swanport and Coles Bay samples. Despite growth in enrichment media, *C. brevifilum* was not successfully established in unialgal culture.

Description.
Two types of scales were observed: spine scales and plate scales (Fig. 4.18).

Spine scales were slightly longer than wide and had dimensions of 0.6 - 0.8 x 0.5 - 0.6 µm ($\bar{x}$=0.72 x 0.55; n=16). A central spine, 0.5 - 0.6 µm long ($\bar{x}$=0.54; n=16), was attached to the base plate by four supporting struts, which did not extend to the scale edge. The base plate was patterned with radiating ridges in quadrants (10 - 14 ridges in each quadrant) overlying very faint concentric fibrils, and had a raised rim, c. 0.07 µm high. On the distal surface of the scale, the radiating ridges extended from the centre to the scale rim, whereas on the proximal surface, the ridges extended right to the scale edge.

Plate scales were oval, 0.7 - 0.9 x 0.6 - 0.7 µm ($\bar{x}$=0.79 x 0.61; n=7), with similar surface patterning as the spine scales, but having slightly more radiating ridges (15 - 17 in each quadrant), and a marginal band.

A smaller plate scale (0.6 - 0.7 x 0.5 - 0.7 µm), first reported by Birkhead and Pienaar (1994) and confirmed by Moestrup and Thomsen (1995), was not seen in this material. This third type of scale is less numerous than the other two scale types.

There are many scale types resembling those of *C. brevifilum* (Moestrup, 1979, Figs. 8 - 9; Hallegraeff, 1983, Fig. 7; this study, Figs. 4.88, 4.92), and careful examination is required to correctly identify this species.

Scales of *C. brevifilum* from different locations are compared in Table 4.10.

Distribution.

C. brevifilum has been recorded from the UK, Denmark, Norway and South Africa (Moestrup and Thomsen, 1995, and references therein; Dahl et al, 1998). It has also been reported from New Zealand (Rhodes and Burke, 1996) and Canada (Smith and Hobson, 1994); however, no micrographs were published to confirm species identification. Another report from New Zealand (Moestrup, 1979), in which a micrograph was published, showed that the species was not actually *C. brevifilum*, but *C. kappa* (Birkhead and Pienaar, 1994). There has been one report from Australia (Hallegraeff, 1983), but again this species is unlikely to be *C. brevifilum,* as spine scales were generally smaller (0.4 - 0.5 μm, spine length 0.3 μm) with fewer radiating ridges (c. 36) and lacked an upright rim.

Toxicity.

Parke et al (1955) stated that *C. brevifilum* was non-toxic to fish. However, *C. brevifilum* was one of five *Chrysochromulina* species implicated in fish kills in Danish coastal waters, resulting in the death of several tonnes of farmed rainbow trout (Knipschildt, 1992). Old cultures of *C. brevifilum* were found to be toxic to the bryozoan, *Electra pilosa* (Jebram, cited in Moestrup, 1994), although cultures were non-toxic when fed to *Artemia* nauplii (Simonsen and Moestrup, 1997). *C. brevifilum* may produce toxins only under certain environmental conditions or alternatively, as it is a phagotrophic species (Jones et al, 1993, 1995), it may become toxic only after ingesting toxic bacteria. It was not tested for toxicity in this study.

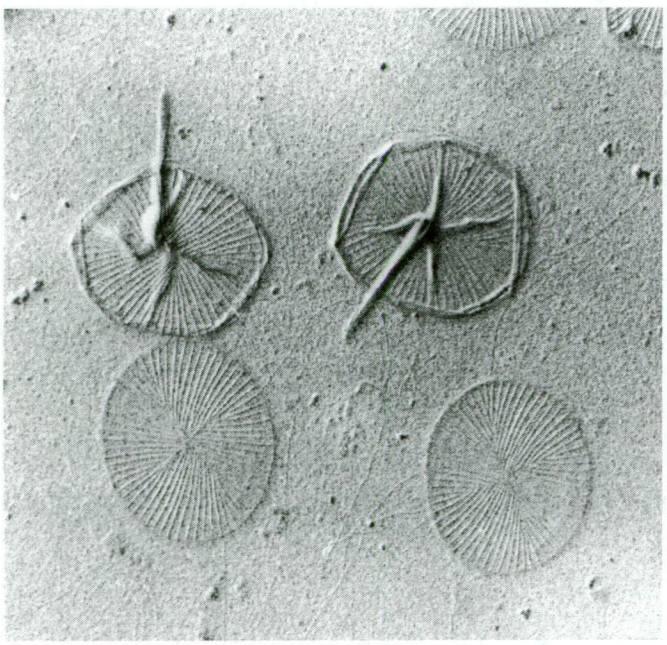


Fig. 4.18: *C. brevifilum* spine scales (0.7 x 0.6 μm) - proximal and distal views; and plate scales (0.7 x 0.6 μm) - distal view; from Fleurty Point

(Micrograph no: 4972)

Table 4.10: Scales of *Chrysochromulina brevifilum* from different locations.

SOURCE	SPINE SCALES				PLATE SCALES		
	Dimensions (µm)	*Spine Height (µm)*	*Rim Height (µm)*	*No. of Ridges*	*Dimensions (µm)*	*Rim Width (µm)*	*No. of Ridges*
UK (Parke et al, 1955)	0.7	0.7	ND	ND	-	-	-
South Africa (Birkhead & Pienaar, 1994)	0.84 - 0.98 x 0.75 - 0.89 ($\bar{x}$ =0.9 x 0.8)	0.63 - 0.72	0.075	58 - 62	(1) 0.76 - 1.02 x 0.68 - 0.92 ($\bar{x}$ =0.9 x 0.72)	0.07	58 - 64
					(2) 0.6 - 0.71 x 0.53 - 0.66 ($\bar{x}$ =0.7 x 0.6)	ND	44 - 48
Denmark (Moestrup, 1995)	0.63 - 0.98 x 0.55 - 0.84	0.6 - 0.7	c. 0.08	c. 60	(1) 0.76 x 1.02 - 0.68 x 0.92	0.07 - 0.10	c. 68
					(2) 0.57 - 0.71 x 0.52 - 0.66	c. 0.08	c. 48 - 52
Tasmania, Australia	0.6 - 0.8 x 0.5 - 0.6 ($\bar{x}$ =0.72 x 0.55; n=16)	0.5 - 0.6 ($\bar{x}$ =0.54; n=16)	c. 0.07 (n=7)	40 - 56 (n=11)	(1) 0.7 - 0.9 x 0.6 - 0.7 ($\bar{x}$ =0.79 x 0.61; n=7)	0.08 - 0.09 ($\bar{x}$ =0.08; n=6)	60 - 68 (n=3)
					(2) -	-	-

ND = Not determined

***Chrysochromulina* aff. *camella* Leadbeater et Manton** **Fig. 4.19**

Micrographs: Leadbeater and Manton, 1969; Fig. 7.
Moestrup, 1979; Fig. 12.
Rhodes and Burke, 1996; Fig. 6C.

Present Findings.
A small scale cluster was found in an off-shore sample from Pirates Bay.

This is a new record for Australian waters.

Description.
Scales were similar to the cup scales of *C. camella* (Leadbeater and Manton, 1969). They had a cup-like shape, patterned with rows of rectangular perforations, with a wide layered rim (Fig. 4.19). However, these scales were larger than those of *C. camella* (Table 4.11); they had only two rows of rectangular perforations instead of four; and they appeared to have a wider base.

Scales from New Zealand, described by Rhodes and Burke (1996) as *C. camella,* were also slightly larger than the type material (Table 4.11), but otherwise agreed with the type description.

Only two other species of *Chrysochromulina* have cup-like scales: *C. strobilus* and *C. cymbium* (Leadbeater and Manton, 1969). These scale types have longitudinal striations instead of rows of rectangular perforations.

Distribution.
C. camella was originally described from the English Channel (Leadbeater and Manton, 1969), and has since been reported only from New Zealand coastal waters (Moestrup, 1979; Rhodes and Burke, 1996).

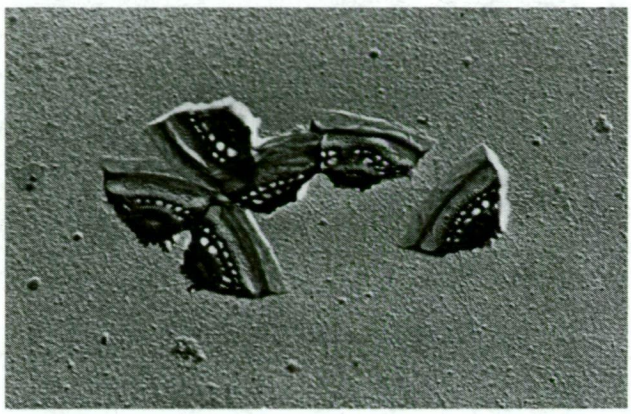


Fig. 4.19: *C.* aff. *camella* cup scales (0.7 μm width and 0.4 μm height); from Pirates Bay

(Micrograph no: 5105)

Table 4.11: *Chrysochromulina camella* cup scales from different locations.

SOURCE	WIDTH AT RIM (μm)	HEIGHT (μm)	NO. OF PERFORATED ROWS
Chrysochromulina camella			
UK *(type)* (Leadbeater & Manton, 1969)	0.20	0.25	4
New Zealand (Rhodes & Burke, 1996)	0.35 - 0.40 ($\bar{x}$ =0.38; n=4)	0.25 - 0.30 ($\bar{x}$ =0.27; n=4)	4
Chrysochromulina aff. camella			
Tasmania, Australia	0.67 - 0.75 ($\bar{x}$ =0.70; n=6)	0.33 - 0.42, ($\bar{x}$ =0.39; n=6)	2

***Chrysochromulina chiton* Parke et Manton** **Figs. 4.20 - 4.22**

Micrographs: Parke et al, 1958; Figs. 15 - 17.
Manton, 1967; Figs. 3 - 4.
Manton, 1968; Fig. 4.
Leadbeater 1972b; Figs. 26 - 29.
Beech, 1983; Plate 2.2E.

Present Findings.
Scales were found in samples from Pipeclay Lagoon.

Description.
Two types of scales were seen (Fig. 4.20), with the smaller plate scales having a variable size range.

The larger scale type had a distinctive oval "hat-like" shape (1.4 - 1.6 x 1.1 - 1.2 μm; $\bar{x}$=1.5 x 1.2 μm; n=13), with a central oval area separated from the rim (or "brim"!) by a distinct raised ridge (Fig. 4.20). Both the central area and the rim were patterned with very fine radiating ridges, c. 100, arranged in quadrants.

The second scale type was also oval, but smaller (0.5 - 0.8 x 0.4 - 0.7 μm; $\bar{x}$=0.7 x 0.6 μm; n=12), with a narrow raised perforated rim. Scales were patterned on one surface with radiating ridges (c. 50 - 60 in quadrants), extending right to the scale edge, and superimposed on faint concentric fibrils. The opposite surface had an extremely faint pattern of radiating ridges which extended to a marginal band (Fig. 4.21). This patterning was seen on all plate scales, regardless of size (Fig. 4.22). However, Manton (1967) found scales from cultured material with the same pattern of radiating ridges on both scale surfaces. It has been suggested that the two forms of scales are representative of cells from different life cycle stages (Eikrem and Edvardsen, 1999).

Scales observed in this study matched those of the type description (Parke et al, 1958), although sizes were generally smaller (Table 4.12). Leadbeater (1972b) noted that *C. chiton* scale size is extremely variable and this is demonstrated in later records of the species (Table 4.12).

Another type of scale similar to that of *C. chiton,* but lacking the broad patterned rim and with fewer radiating ridges, was also found in this survey. This scale resembled scales described as *C. chiton* by Hallegraeff (1983; Fig. 6) and Moestrup (1979; Fig. 10) from Australian and New Zealand waters respectively (Table 4.12).

Distribution.

C. chiton is widely distributed and has been reported from England, Norway Denmark, the Mediterranean Sea and the North Atlantic Ocean in the northern hemisphere, and from New Zealand and Australia in the southern hemisphere (Beech, 1983; Estep et al, 1984, and references therein; Leadbeater 1974; Manton and Leadbeater, 1974). A new variety of *C. chiton*, *C. chiton* var. *minuta*, has been described from Jiaozhou Bay, China (Gao et al, 1993).

Records have generally been from coastal waters with only one oceanic record (Estep et al, 1984).

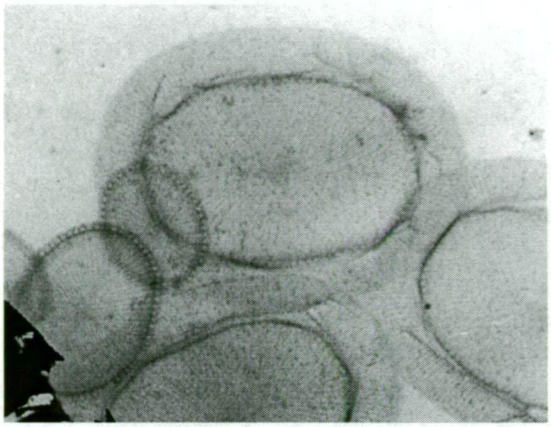


Fig. 4.20: *C. chiton* large "hat-like" scales (1.5 x 1.2 μm); and plate scales (2 sizes); from Pipeclay Lagoon

(Micrograph no: 5209)

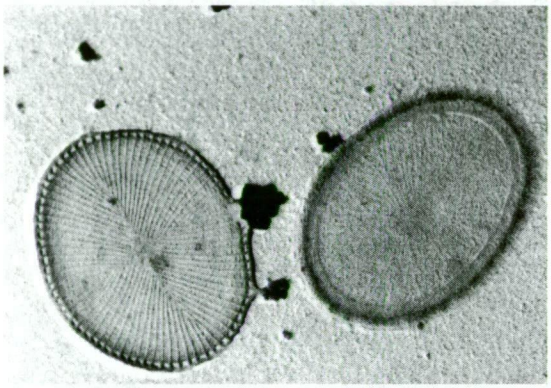


Fig. 4.21: *C. chiton* plate scales (0.7 x 0.6 μm) - proximal and distal views; from Pipeclay Lagoon

(Micrograph no: 5209)

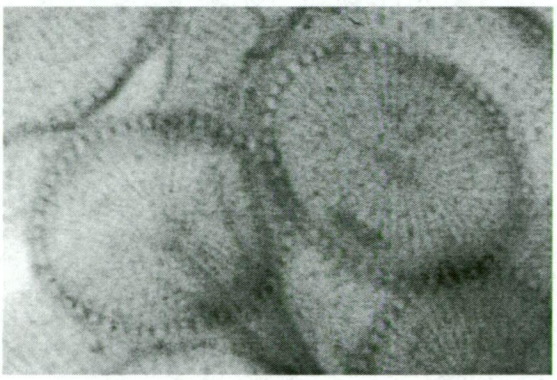


Fig. 4.22: *C. chiton* small plate scales (0.5 x 0.4 μm) showing same surface patterning as larger plate scales; from Pipeclay Lagoon

(Micrograph no: 5209)

Table 4.12: Scales of *Chrysochromulina chiton* compared with those of *C.* aff. *chiton* from different locations.

SOURCE	LARGE SCALES				SMALL SCALES		
	Length (µm)	*Width (µm)*	*Rim Width (µm)*	*No. of Ridges*	*Length (µm)*	*Width (µm)*	*No. of Ridges*
Chrysochromulina chiton							
England *(type)* (Parke et al, 1958)	2.4 - 2.9	1.9 - 2.2	0.3 (n=5)	c. 100 (n=5)	0.9 - 1.4	0.7 - 1.1	c. 50 (n=2)
Norway (Leadbeater, 1972)	1.5 (n=1)	1.2 (n=1)	0.15 (n=2)	c. 100 (n=2)	0.7 - 0.9 (n=2)	0.6 - 0.7 (n=2)	c. 60 (n=2)
N. Atlantic Ocean (Estep et al, 1984)	2.4	2.0	-		1.2	0.9	-
Victoria, Australia (Beech, 1983)	2.2	1.4	0.1 (n=1)	c. 100 (n=1)	-	-	-
Tasmania, Australia	1.4 - 1.6 ($\bar{x}$ =1.5; n=13)	1.1 - 1.2 ($\bar{x}$ =1.2; n=13	0.2 (n=12)	c. 100 (n=12)	0.5 - 0.8 ($\bar{x}$ =0.7; n=12)	0.4 - 0.7 ($\bar{x}$ =0.6; n=12)	c. 60 (n=2)
Chrysochromulina* aff. *chiton							
New Zealand (Moestrup, 1979)	1.3	1.0	0.08 (n=1)	c. 80 (n=1)	0.7	0.5	c. 40 (n=1)
New South Wales, Australia (Hallegraeff, 1983)	1.2	0.8	0.08 (n=1)	c. 70 (n=1)	0.7	0.5	c. 50
Tasmania, Australia	1.1 (n=1)	0.8 (n=1)	0.08 (n=1)	c. 80 (n=1)	-	-	-

***Chrysochromulina cyathophora* Thomsen** **Figs. 4.23 - 4.24**

Micrographs: Thomsen, 1979; Figs. 3 - 4.
Manton, Oates and Sutherland, 1981; Fig. 3.

Present Findings.
One whole cell and several scales were found in the Southport sample.

Description.
Numerous cylinder scales surrounded the cell (c. 2.5 µm diameter) which had a partially coiled haptonema (6 µm) and one smooth flagellum (5 µm) (Fig. 4.23), the other flagellum presumably being lost during sample preparation.

Cylinder scales are characteristic of three *Chrysochromulina* species: *C. megacylindra*, *C. microcylindra*, and *C. cyathophora* (Leadbeater, 1972a; Thomsen, 1979). There were sufficient differences between these species to identify the Tasmanian material as *C. cyathophora* on the basis of cell size, flagellar and haptonemal length, and scale type (Table 4.13).

Cylinder scales were thin-walled and straight-sided, with length 0.6 - 0.7 µm ($\bar{x}$=0.64; n=7) and width 0.2 - 0.3 µm ($\bar{x}$=0.26; n=7) (Fig. 4.24). These dimensions were significantly smaller than those of *C. megacylindra* and *C. microcylindra*, but similar to those of *C. cyathophora* (Table 4.13). Thomsen (1979) reported in the species description of *C. cyathophora* that there is an abrupt decrease in cylinder-wall thickness at about two-thirds from the base, but this was not observed in the Tasmanian material.

The base plate of the cylinder appeared rectangular, and was slightly narrower than the width of the cylinder scale, having a diameter of 0.12 - 0.14 µm (n=3) (Fig. 4.24). This scale matched the type material, although the radiating ridges on the proximal surface as described by Thomsen (1979; Figs. 3A, 4A) were not seen. In contrast, the cylinder scale base-plates of *C. microcylindra* and *C. megacylindra* are much larger, being at least twice the width of their respective cylinder scales, and are distinctly patterned with radial striations (Leadbeater, 1972a; Plates 1, 2).

Approximately 30 cylinder scales formed an outer layer surrounding the cell (Fig. 4.23), which was far fewer than Thomsen's record of 125 scales. Other outer scales may have been lost during sample preparation.

No individual plate scales were observed in the Tasmanian material, in contrast to *C. microcylindra* and *C. megacylindra* which have obvious plate scales as well as cylinder scales (Leadbeater, 1972a, Plates 1 and 2; Manton et al, 1981, Fig. 2). However, a clearly defined dark area around the cell perimeter (Fig. 4.23) indicated that there was an inner scale case, possibly formed by these plate scales, as described by Thomsen (1979). Thomsen also noted that the plate scales are only rarely seen and have a characteristic pattern of concentric ridges, quite distinct from the patterning on plate scales of *C. megacylindra* and *C. microcylindra.*

Distribution.

C. cyathophora has been reported from Denmark, at temperatures of 11 - 14°C and salinities of 8 - 17 psu (Thomsen, 1979), South Alaska, at temperatures of 6 - 10°C (Manton et al, 1976; Manton et al, 1981), and Australia (Beech, 1983).

In the present study, *C. cyathophora* was collected from the most southern sampling location, Southport, during summer when the water temperature was 20°C, indicating that it has a wide temperature tolerance.

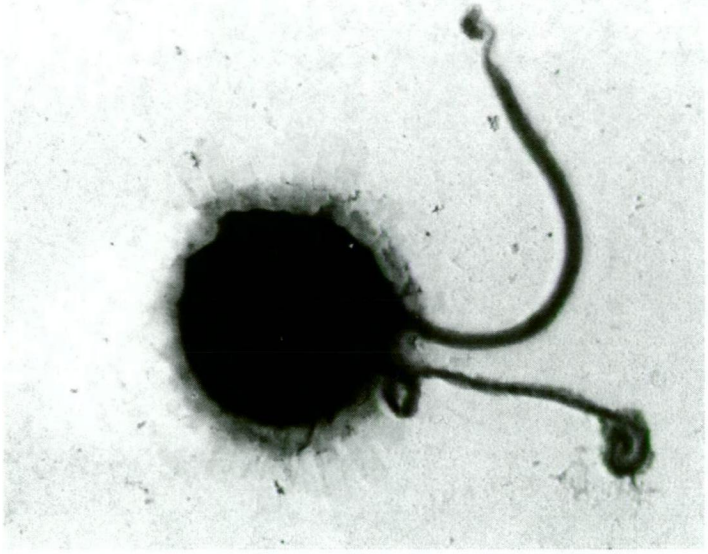


Fig. 4.23: *C. cyathophora* cell (c. 2.5 μm), showing one flagellum, haptonema and scale covering; from Southport

(Micrograph no: 5401)

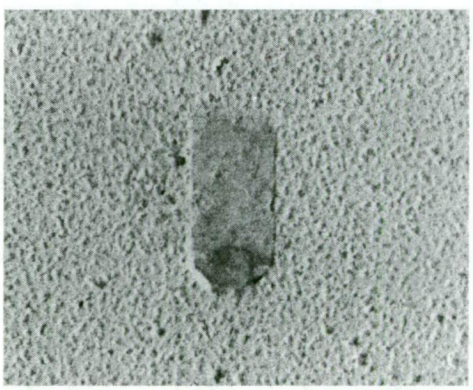


Fig. 4.24: *C. cyathophora* cylinder scale (c. 0.6 μm long); from Southport

(Micrograph no: 5402)

Table 4.13 : Comparison of *Chrysochromulina* species with cylinder scales: *C. cyathophora*, *C. megacylindra* and *C. microcylindra*.

SPECIES/SOURCE	CELL DIAM. (μm)	FLAGELLA (μm)	HAPTONEMA (μm)	CYLINDER SCALES			PLATE SCALES (μm)
				Height (μm)	*Width (μm)*	*Base (μm)*	
C. cyathophora							
Denmark *(type)* (Thomsen, 1979)	c. 3.0 (2.9 - 3.3)	10 - 12	7	0.5 - 1.1	0.2	0.16 - 0.19	c. 0.8
South Alaska (Manton et al, 1981)	-	-	-	0.8 - 1.0 ($\bar{x}$=0.86; n=9)	0.35 - 0.40 ($\bar{x}$=0.38; n=9)	c. 0.2 (n=1)	0.7 - 0.9 (n=2)
Victoria, Australia (Beech, 1983)	-	c. 7	-	0.5 (n=2)	0.2 - 0.3 (n=2)	ND	0.6 - 0.7 (n=2)
Tasmania, Australia	c. 2.5	6	5 (coiling)	0.6 - 0.7 ($\bar{x}$=0.64; n=7)	0.2 - 0.3 ($\bar{x}$=0.26; n=7)	0.12 - 0.14 (n=3)	-
C. megacylindra							
Norway (Leadbeater, 1972)	5 x 4	20	18	1.0	0.5	0.7	1.0
C. microcylindra							
Norway (Leadbeater, 1972)	4 - 7	20 - 25	16 - 30	0.5 - 3.0	0.1 - 0.2	0.4 - 0.5	0.6 - 1.0 x 0.5 - 1.0

ND = Not determined

***Chrysochromulina ephippium* Parke et Manton** **Fig. 4.25**

Micrographs: Parke et al, 1956; Figs. 38 - 40.
Manton and Leadbeater, 1974; Fig. 4.
Beech, 1983; Plate 2.2 C - D.

Present Findings.
Spine scales were found in the Fleurty Point sample.

Description.
Spine scales were circular to oval, 0.36 - 0.41 μm, with a central spine approximately equal to the scale diameter in length, a raised rim, and a base pattern of c. 40 radiating ridges arranged in quadrants. The central spine was supported by four decurrent struts which extended to the scale edge on the proximal side, but were not visible on the distal side (Fig. 4.25). No plate scales were observed.

Spine scales closely matched those of the type species (Parke et al, 1956). Manton and Leadbeater (1974) noted the consistent scale size and patterning in *C. ephippium*, and this has been reflected in later records of this species (Table 4.74).

C. ephippium spine scales may be easily confused with those of *C. acantha* or *C. alifera*, both of which have a similar size and structure (Eikrem and Moestrup, 1998; Table 2). However, *C. acantha* spine scales do not have a raised rim, while those of *C. alifera* have supporting struts which do not extend to the scale margin and a spine length less than the scale diameter.

Distribution.
C. ephippium appears to be widely distributed and has been reported from the UK, Norway, Denmark, Greenland, West Greenland, Yugoslavia, Algiers, Canada, South Africa, Australia, New Zealand and the North Atlantic Ocean (Beech, 1983; Estep et al, 1984, and references therein; Rhodes and Burke, 1996; Smith and Hobson, 1994). Not all these records included published micrographs to confirm species identity.

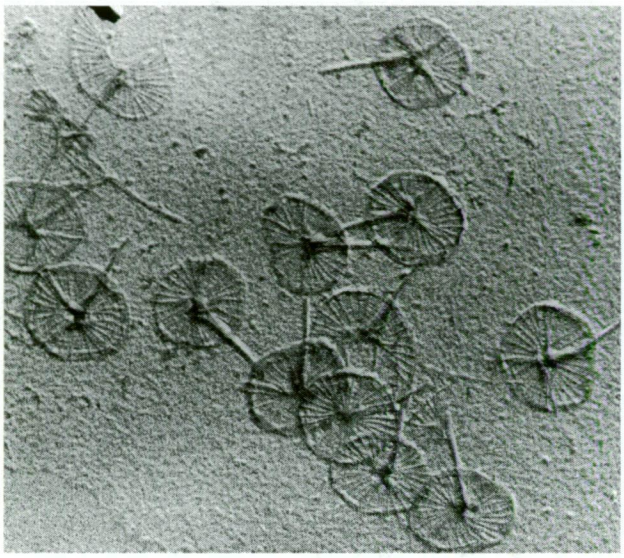


Fig. 4.25: ***C. ephippium*** **spine scales (0.4 µm); from Fleurty Point**

(Micrograph no: 4954)

Table 4.14: *Chrysochromulina ephippium* spine scales from different locations.

SOURCE	DIMENSIONS (μm)	SPINE HEIGHT (μm)	NO. OF RIDGES	RAISED RIM
UK *(type)* (Parke et al, 1956)	0.3 - 0.6	= scale diameter	c. 40	Yes
Denmark (Manton & Leadbeater, 1974)	0.3 - 0.6	≥ scale diameter	c. 40	Yes
North Atlantic Ocean (Estep et al, 1984)	0.3 - 0.5 x 0.4 - 0.6	ND	ND	ND
New Zealand (Moestrup, 1979)	0.4 - 0.9	ND	ND	ND
Victoria, Australia (Beech, 1983)	0.3 x 0.4 - 0.5 (n=2)	0.5 (n=1)	c. 56 (n=2)	Yes
Tasmania, Australia	0.36 - 0.41 ($\bar{x}$ =0.38; n=10)	0.36 - 0.38 ($\bar{x}$ =0.37; n=6)	c. 40 (n=6)	Yes

ND = Not determined

***Chrysochromulina ericina* Parke et Manton** **Figs. 4.26 - 4.29**

Micrographs: Parke et al, 1956; Plates 3 - 4.
Manton and Leedale, 1961; Plate 4.
Leadbeater, 1972b; Plate 3.
Moestrup, 1979; Figs. 20 - 22.
Beech, 1983; Plate 2.3.
Rhodes and Burke, 1996. Fig. 3A.

Present Findings.
Scales were found in samples from the Derwent River, Pipeclay Lagoon and Honey Moon Bay. They were also found in a sample from Ocean Beach on the west coast of Tasmania.

Whole cells and scales were seen in GSe and ML (+GeO_2) enrichment cultures derived from Derwent River. Scales only were found in enrichment cultures from Fleurty Point (GSe medium), Pirates Bay (K/2 medium), Honey Moon Bay (ML medium) and Coles Bay (ML medium).

Description.
Whole cells were rare and poorly preserved, whereas scales were common and well defined. There were two scale types: long cylindrical spine scales and plate scales.

Cylindrical spine scales were "trumpet-like", slightly tapering with a blunt end and a flared conical base (Fig. 4.26). The base (1.0 - 1.5 µm; $\bar{x}$=1.3 µm; n=17) was closed by a flat plate with similar patterning to the plate scales. Faint longitudinal striations were seen along the length of the spine (Figs. 4.27, 4.28) which ranged from 8 - 13 µm ($\bar{x}$=9.8 µm; n=13) with a width of 0.1 - 0.2 µm ($\bar{x}$=0.2 µm; n=15). Manton and Leedale (1961), by studying sections under the transmission electron microscope, found that these cylindrical spines are hollow. Beech (1983) suggested that the blunt end is actually open, but to confirm this, TEM sections would be required.

Plate scales were oval (1.0 - 1.2 x 0.8 - 0.9 µm; $\bar{x}$=1.1 x 0.8 µm; n=11) and rimmed. The proximal surface was patterned with c. 80 slightly curved radiating ridges, arranged in quadrants and extending to the scale rim (Fig. 4.29). The distal surface had a loosely concentric pattern of interwoven fibrils extending to a peripheral band, which was patterned with very fine concentric fibrils (Fig. 4.28).

Scale size, particularly cylindrical spine length, was extremely variable (Leadbeater, 1972b), regardless of whether the material examined was from wild samples or laboratory cultures (Table 4.15).

Distribution.

C. ericina has been reported from coastal waters in both northern and southern hemispheres, including those of: UK (Parke et al, 1956), Canada (Smith and Hobson, 1994), Norway (Leadbeater, 1972b, and references therein), Denmark (Manton and Leadbeater, 1974; Knipschildt, 1992), Yugoslavia, Algeria (Leadbeater, 1974), New Zealand (Moestrup, 1979; Rhodes and Burke, 1996) and south-east Australia (Beech, 1983).

Toxicity.

Parke et al (1956) reported that *C. ericina* was non-toxic to fish. However, it was one of five species present in a *Chrysochromulina* bloom that resulted in the death of several tonnes of rainbow trout in Danish coastal waters (Knipschildt, 1992; Hansen et al, 1994)

C. ericina was found to be non-toxic to *Artemia* nauplii (Edvardsen and Paasche, 1992; Rhodes and Burke 1996; Simonsen and Moestrup, 1997) even when grown in phosphate-deplete medium (Edvardsen 1993).

This species was not cultured in the present work and hence was not tested for toxicity.

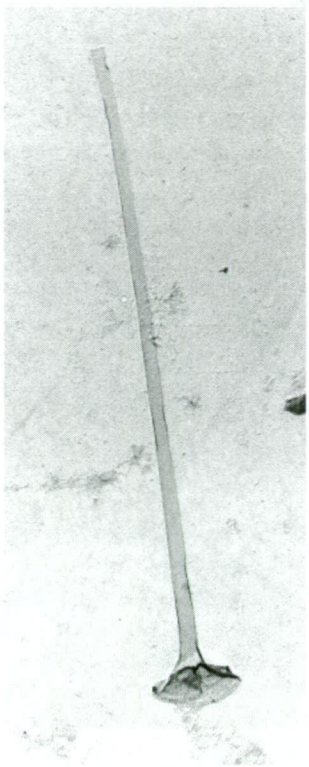


Fig 4.26: *C. ericina* spine scale (10 μm long)

(Micrograph no: 5090)

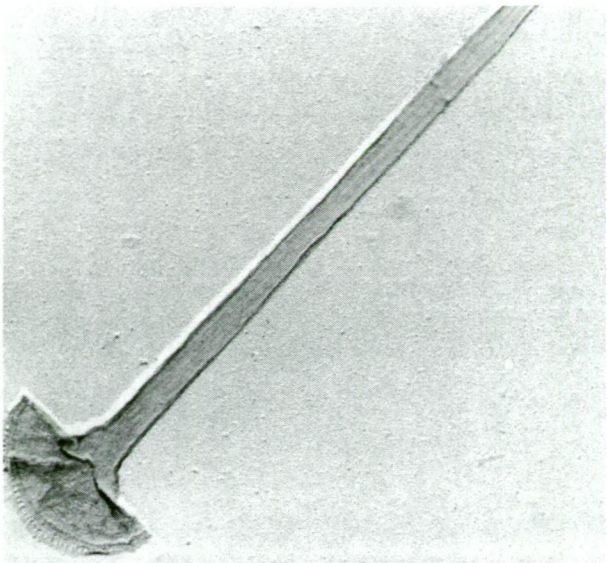


Fig. 4.27: *C. ericina* spine scale showing detail of base; from a Derwent enrichment culture

(Micrograph no: 5089)

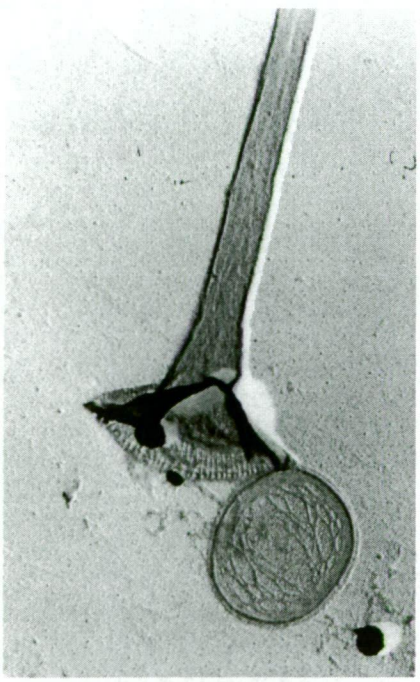


Fig. 4.28: *C. ericina* spine scale and plate scale (c. 1 μm) - distal view; from a Derwent enrichment

(Micrograph no: 5072)

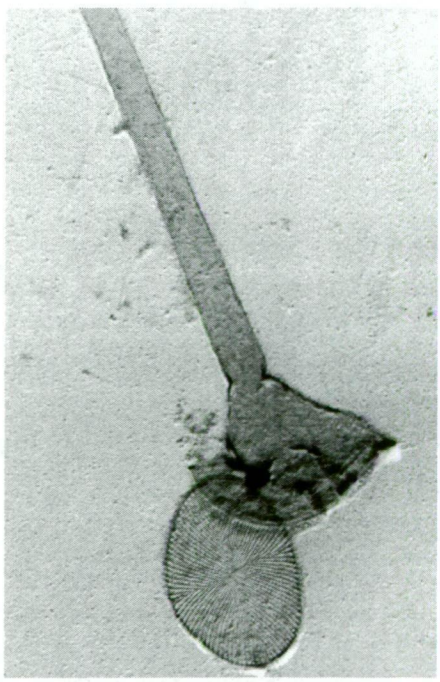


Fig. 4.29: *C. ericina* spine scale and plate scale (1.0 x 0.8 μm) - proximal view; from a Derwent enrichment

(Micrograph no: 5072)

Table 4.15: Scale sizes of *Chrysochromulina ericina* from different locations.

SOURCE	MATERIAL EXAMINED	PLATE SCALES		CYLINDRICAL SPINE SCALES		
		Length (μm)	*Width* (μm)	*Base Diameter* (μm)	*Spine Height* (μm)	*Spine Width* (μm)
UK *(type)* (Parke et al, 1956)	Cultured	0.6 - 0.9	0.5 - 0.7	1 - 1.4	9 - 15	0.2 - 0.3
Norway (Leadbeater, 1972)	Wild	1.2 -1.3	ND	0.6 - 2.7	1.4 -14	0.3 - 1.4
New Zealand Moestrup, 1979	Wild	1.2	0.6	1.3 (n=1)	ND	0.2 (n=1)
New Zealand (Rhodes & Burke, 1996)	Cultured	0.9 - 1.2	0.7 - 0.9	1.4 - 1.7	9 - 16.4	0.1 - 0.3
Victoria, Australia (Beech, 1983)	Wild	1.2 - 1.4 ($\bar{x}$ =1.3; n=5)	0.8 - 1.0 ($\bar{x}$ =0.9; n=5)	1.9 (n=1)	18 (n=1)	0.3 (n=1)
Tasmania, Australia	Cultured	1.0 - 1.2 ($\bar{x}$ =1.1; n=11)	0.8 - 0.9 ($\bar{x}$ =0.8; n=11)	1.0 - 1.5 ($\bar{x}$ =1.3; n=17)	8 - 13 ($\bar{x}$ =9.8; n=13)	0.1 - 0.2 ($\bar{x}$ =0.2; n=15)

ND = Not determined

***Chrysochromulina fragaria* Eikrem et Edvardsen** **Figs. 4.30 - 4.32**

Micrographs: Beech, 1983; Plate 2.10 C - D.
Eikrem and Edvardsen, 1999; Figs. 1 - 5.

Present Findings.
Scales were observed in samples from Dru Point, Oyster Cove Point, Deep Bay and the Derwent River. Whole cells were seen in the Deep Bay and Derwent River samples.

Description.
Cells were approximately 4 µm and had two flagella (11 and 13 µm) and a short haptonema (Fig. 4.30).

Two typical *Chrysochromulina* scale types were seen. One type had a distinctive high rim, giving the scale a polygonal shape (usually square or pentagonal). The rim was unpatterned and had a height ranging from 0.08 - 0.16 µm ($\bar{x}$=0.12; n=8). The scale itself was oval to circular with dimensions of 0.58 - 0.77 x 0.53 - 0.76 µm ($\bar{x}$=0.69 x 0.63 µm; n=14). Proximal and distal surfaces had a pattern of c. 60 radiating ridges arranged in quadrants (Figs. 4.31, 4.32).

The other scale type was a circular to oval plate scale, 0.65 - 0.81 x 0.56 - 0.67 µm ($\bar{x}$=0.73 x 0.60 µm; n=13), patterned with c. 80 - 90 radiating ridges arranged in quadrants. On one surface, these ridges extended from the centre to the scale edge; on the opposite surface, they extended to a broad rim (0.09 µm; n=1) with faint concentric striations (Fig. 4.32).

This species closely matched the type description (Eikrem and Edvardsen, 1999), and also the description given by Beech (1983) of material from Australian coastal waters (Table 4.16). Another species having similar cell and scale morphology, isolated from Canada and maintained in the Provasoli-Guillard National Centre for Culture of Marine Phytoplankton (as CCMP-1204), was found to be genetically distinct from *C. fragaria* (Eikrem and Edvardsen, 1999).

Distribution.

C. fragaria was originally described from Norway and has also been recorded from Denmark and the Baltic Sea (Eikrem and Edvardsen, 1999, and references therein). It was also reported by Beech (1983) from south-east Australia as *Chrysochromulina* sp. 3.

Toxicity.

C. fragaria has been found to be non-toxic to *Artemia* nauplii (Eikrem and Edvardsen, 1999). It was a dominant species in the 1995 *Chrysochromulina* bloom in the Skagerrak (Norway) which caused minor fish mortalities; however, *C. polylepsis*, a known toxic species, was also dominant in this bloom (Dahl et al, 1998; Edvardsen and Paasche, 1998).

This species was not cultured in the present work and hence unable to be tested for toxicity.

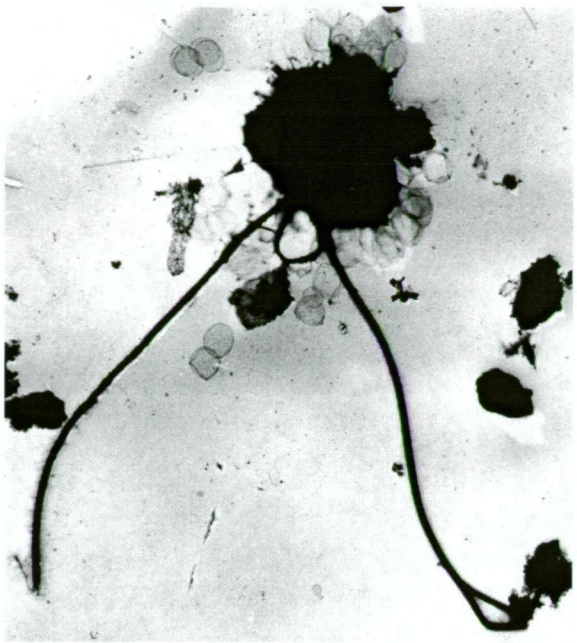


Fig. 4.30: *C. fragaria* cell (c. 4 μm); showing two flagella and short haptonema; from Deep Bay

(Micrograph no: 4991)

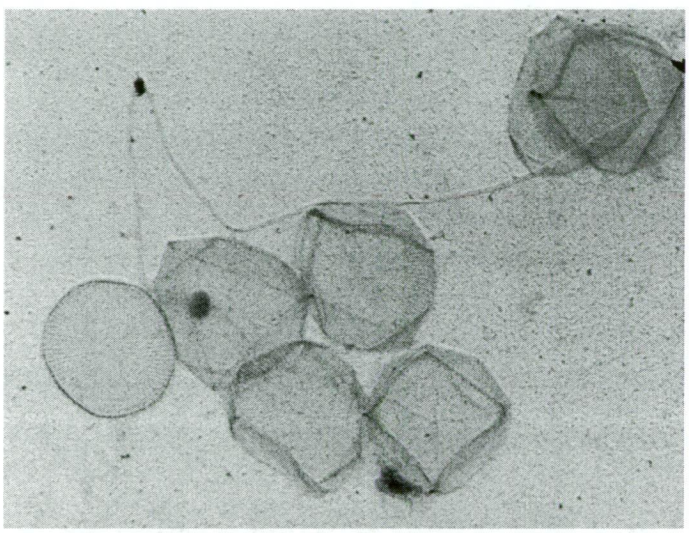


Fig. 4.31: *C. fragaria* rimmed scales and plate scales (0.7 x 0.6 μm); from the Derwent River

(Micrograph no: 5182)

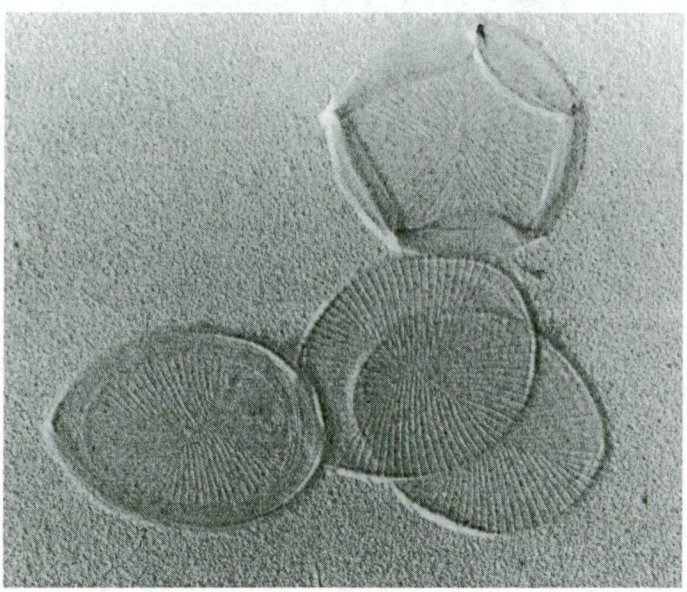


Fig. 4.32: *C. fragaria* plate scales - proximal and distal views; and one rimmed scale (rim height 0.12 μm); from the Derwent River

(Micrograph no: 4890)

Table 4.16: *Chrysochromulina fragaria* from different locations.

SOURCE	CELL SIZE (µm)	FLAGELLA LENGTH (µm)	HAPTONEMA LENGTH (µm)	RIMMED PLATE SCALES				PLATE SCALES			
				Length (µm)	*Width (µm)*	*Rim Height (µm)*	*No. of Ridges*	*Length (µm)*	*Width (µm)*	*Rim Width (µm)*	*No. of Ridges*
Norway *(type)* (Eikrem & Edvardsen, 1999)	4 - 8	10 - 14	3 - 8	0.5 - 0.8	0.5 - 0.8	0.15 - 0.20	c. 75 - 90	0.45 - 0.8	0.35 - 0.75	0.05 - 0.1	c. 65 - 90
Victoria, Australia (Beech, 1983)	5	20	10	0.4 - 0.6	0.4 - 0.6	0.12 (n=2)	c. 90	0.4 - 0.6	0.4 - 0.6	0.06 (n=2)	c. 70
Tasmania, Australia	4	11 - 13	ND	0.58 - 0.77 ($\bar{x}$ =0.69; n=14)	0.53 - 0.76 ($\bar{x}$ =0.63; n=14)	0.08 - 0.16 ($\bar{x}$ =0.12; n=8)	c. 60	0.65 - 0.81 ($\bar{x}$ =0.73; n=13)	0.56 - 0.67 ($\bar{x}$ =0.60; n=13)	0.09 (n=1)	c. 80 - 90

ND = Not determined

***Chrysochromulina hirta* Manton** **Figs. 4.33 - 4.35**

Micrographs: Manton, 1978a; Figs. 8 - 17.
Beech, 1983; Plate 2.4A.
Rhodes and Burke, 1996; Fig. 4.

Present Findings.

Chrysochromulina hirta was one of the most commonly observed species in this survey. It was found at nine sites: the Derwent River, all Channel sites, Deep Bay, Southport, Pipeclay Lagoon and Roches Beach, at temperatures ranging from 12 - 20°C. Spine scales were common, but few whole cells were seen.

C. hirta grew well in a range of enrichment media. Scales and whole cells were found in enrichment cultures derived from the Derwent River (GSe, GSe(+GeO_2), ML media), Storm Bay (GSe/2 and ML media), Fleurty Point (GSe medium) and Southport (ML medium). Scales were found in enrichment cultures from Pirates Bay (f-Si/100 and K/10 media), Honey Moon Bay (GSe medium) and Coles Bay (ML medium). It was unlikely that these scales had been transferred to cultures in initial inocula as they were present in high numbers, indicating that live cells had grown.

C. hirta was successfully isolated by micromanipulation from a Derwent River enrichment culture. A unialgal culture (CS-482) is currently maintained in the CSIRO Collection of Living Microalgae (in GSe medium at 15°C, under standard growth conditions).

Description.

Whole cells were about 6 µm in diameter, with two equal flagella, 11 - 13 µm in length, and a scale covering of three different types. Unfortunately, the haptonema was not found attached to the cells and was most likely lost during sample preparation.

Two different types of spine scales and one type of plate scale characterised this species. Large spine scales had a tapering, twisted spine, 9 - 17 µm in length ($\bar{x}$=11 µm; n=26), attached to a concave base (1.6 - 2.3 µm diameter; $\bar{x}$=1.9 µm; n=10) and supported by four obvious struts, each with a thread-covered surface (Fig. 4.33).

Small spine scales ("umbrella scales") had a shorter, thinner spine, 3 - 5 μm in length ($\bar{x}$=4.4 μm; n=25), which was also attached to a concave base, 1.2 - 2.2 μm in diameter ($\bar{x}$=1.8 μm; n=17), and supported by four smaller and less obvious struts (Fig. 4.34).

In both cases, the concave base was patterned with radiating ridges and had a thickened rim. Small spine scales were more numerous than the larger ones.

Plate scales were circular to oval, with dimensions 0.8 - 1.5 x 0.8 - 1.4 μm ($\bar{x}$=1.3 x 1.1 μm; n=7). These thin scales had a characteristic pattern of radiating ridges (c. 80 - 90) arranged in quadrants and extending to the scale edge on the proximal side, and concentric fibrils with superimposed irregular radial threads on the distal side (Fig. 4.35).

Scale structure matched that of the type description (Manton, 1978a). Comparing scale sizes with the type material, and with material described from Australia (Beech, 1983) and New Zealand (Rhodes and Burke, 1996), it was found that plate scales and small spine scales were of similar size, while large spine scales from later records were considerably smaller than the original material (Table 4.17).

Distribution.

C. hirta has been reported from Denmark, Alaska (Manton, 1978a; Knipschildt, 1992) and Canada (Smith and Hobson, 1994) in the northern hemisphere, and from South Africa (Manton, 1978a), south-east Australia (Beech, 1983), New Zealand (Rhodes and Burke, 1996) and the Southern Ocean (Marchant, 1993) in the southern hemisphere. It has a wide temperature tolerance ranging from -1°C to 20°C.

Toxicity.

C. hirta was found to be non-toxic to *Artemia* nauplii (Edvardsen and Paasche, 1992; Edvardsen 1993; Simonsen and Moestrup, 1997), which agreed with findings in this study (see Chapter 7).

However, *C. hirta* was one of five species in a dense 1992 *Chrysochromulina* bloom (up to 4.5 x 10^7 cells L^{-1}) in Danish coastal waters, which resulted in the death of several tons of farmed rainbow trout (Knipschildt, 1992; Hansen et al, 1995). It is possible that the long spines of *C. hirta* and two other species involved in the bloom, *C. ericina* and *C. spinifera*, caused sufficient gill damage to result in fish mortality.

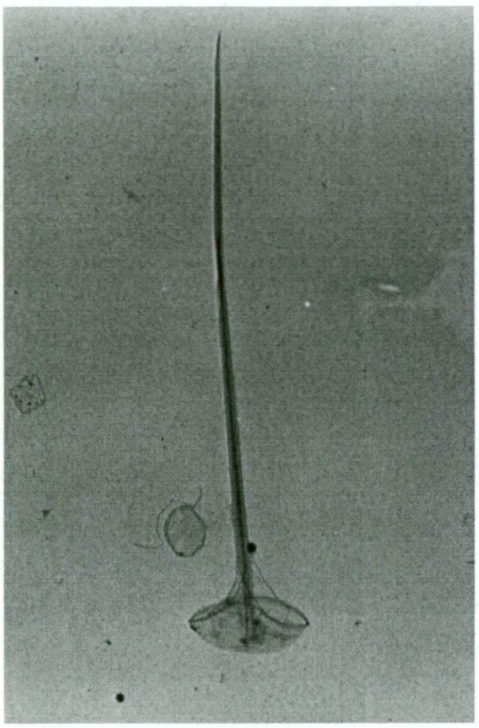


Fig. 4.33: *C. hirta* large spine scale (2 μm diam.); from a Coles Bay enrichment culture

(Micrograph no: 5462)

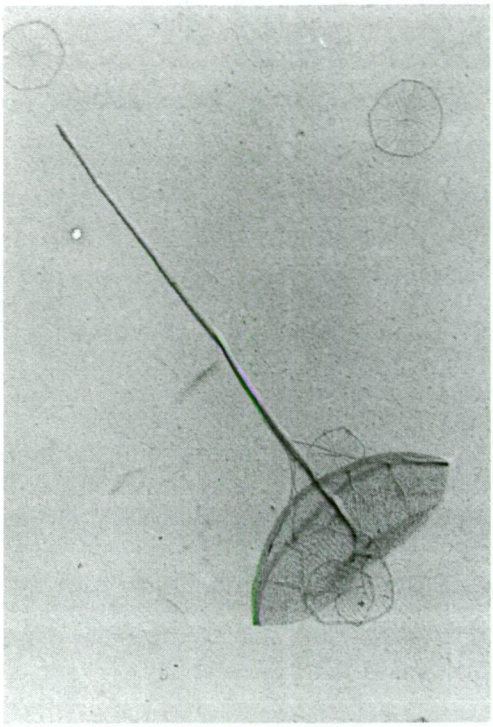


Fig. 4.34: *C. hirta* "umbrella" spine scale (1.2 μm diam.); from a Fleurty Point enrichment culture

(Micrograph no: 5154)

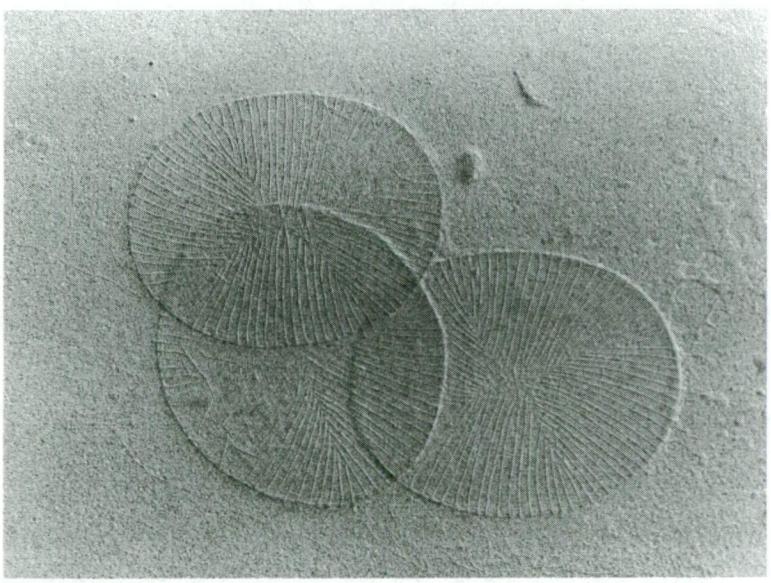


Fig. 4.35: *C. hirta* plate scales (1.3 x 1.0 μm); from a Derwent River enrichment culture

(Micrograph no: 4788)

Table 4.17: *Chrysochromulina hirta* from different locations.

SOURCE	CELL SIZE (μm)	FLAGELLA (μm)	HAPTONEMA (μm)	PLATE SCALES	SMALL SPINES		LARGE SPINES	
				Dimensions (μm)	*Diameter (μm)*	*Height (μm)*	*Diameter (μm)*	*Height (μm)*
South Africa *(type)* (Manton, 1978)	c. 6 - 12	> 20	< 20	1.6 x 1.3	1.5 - 2.0	< 5	2 - 2.5	20 - 30
Victoria, Australia (Beech, 1983)	ND	ND	ND	1.2 x 0.9 (n=2)	1.5 (n=1)	3 - 4 (n=3)	1.6 (n=1)	13
New Zealand (Rhodes & Burke, 1996)	6 - 12	20	c. 20	1.2 x 0.8	2.1 (n=1)	2.4 - 4.7	1.6 (n=1)	11
Tasmania, Australia	c. 6	11 - 13	ND	0.8 - 1.5 x 0.8 - 1.4 ($\bar{x}$ = 1.3 x 1.1; n=7)	1.2 - 2.2 ($\bar{x}$ =1.8; n=17)	3 - 5 ($\bar{x}$ =4.4; n=25)	1.6 - 2.3 ($\bar{x}$ =1.9; n=10)	9 - 17 ($\bar{x}$ =11; n=26)

ND = Not determined

***Chrysochromulina leadbeateri* Estep, Davis, Hargraves et Sieburth**

Figs. 4.36 - 4.37

Micrographs: Leadbeater, 1972b; Plate 1, Figs. 3 - 5.
Hallegraeff, 1983; Fig. 16.
Estep et al, 1984; Figs. 6 - 7.
Hoepffner and Haas, 1990; Figs. 9 - 10.
Eikrem and Throndsen, 1993; Figs. 10 - 11.
Throndsen et al, 1995; Figs. 6 - 7.
Hadju et al, 1996; Fig. 3B.
Eikrem and Throndsen, 1998; Figs. 2 - 5.

Present Findings.
Scales were found in Pipeclay Lagoon and Southport samples.

Description.
Chrysochromulina leadbeateri has two scale types, both of which were seen in the Tasmanian material.

The first scale type was distinguished by an angular upright rim and a patternless surface (Fig. 4.36). Scales of this type usually have a central cross surrounded by a patternless circular area (Estep et al, 1984); however, scales with a totally patternless surface were reported from the Mediterranean Sea by Eikrem and Throndsen (1998; Figs. 18, 21D). Scales were circular to elliptical and ranged from 0.25 - 0.35 µm in size ($\bar{x}$=0.29 µm, n=10).

The second scale type was circular, approximately 0.35 µm diameter (n=2), and lacked an upright rim (Fig. 4.36). It had a distinct pattern of four concentric rings, dividing the scale into three separate bands, and c. 30 evenly-spaced radiating ribs. A central cross was surrounded by a raised concentric ring, 0.1 µm diameter, and an inner band of 13 - 15 circular pores. The middle band consisted of 34 - 36 rectangular pores, and the outer band of 30 - 32 small, almost square, pores.

Scales similar to those of *C. leadbeateri*, but previously undescribed, were found in a Pipeclay Lagoon sample (Fig. 4.37). Two types of scales were seen. Scales with an angular upright rim (c. 0.04 µm in height) had a size range of 0.23 - 0.27 x 0.18 - 0.20 µm ($\bar{x}$=0.25 x 0.19 µm; n=5), which was slightly smaller than the other Tasmanian scales. A central cross was surrounded by a circular patternless area;

from this area, 22 - 28 radiating ridges, superimposed on concentric fibrils, extended to the scale rim.

Scales lacking an upright rim were also slightly smaller, 0.23 x 0.20 μm (n=1), and were less elaborate in surface patterning, having c. 30 evenly-spaced radiating ridges superimposed on concentric fibrils and a central cross.

C. leadbeateri scales vary in dimensions from 0.2 - 0.4 μm (Tables 4.18, 4.19). Other size variations include: the diameter of the inner concentric ring; band widths and pore dimensions; and the width of the upright rim. These can change the overall appearance of scales. For example, broad upright rims, as shown by Hoepffner and Haas (1990; Figs. 9 - 10), give scales a distinct cup-like shape.

Scales also show considerable variation in structure (Eikrem and Throndsen, 1998; Fig. 21). In the rimless scales, the number of prominent concentric rings varies from three to four, while the rimmed scales have a range of surface patterning.

As scale morphology is regarded as a species-specific characteristic, the range of *C. leadbeateri* scale morphologies suggests that this species should be re-examined, and possibly subdivided at the species or subspecies level. Also, the fact that two sets of completely different scale types were recorded from the one Pipeclay Lagoon sample indicates that morphological variation is not related to environmental factors, and thus provides additional evidence for the existence of more than one species.

Distribution.
C. leadbeateri is widely distributed and has been reported in coastal and oceanic waters from sub-arctic to sub-tropical areas.

C. leadbeateri was initially described from Norway (Leadbeater, 1972b) and has since been recorded from: Australian inshore and offshore waters (Beech, 1983; Hallegraeff, 1983); pelagic waters of the southern North Atlantic Ocean and the south Florida coast (Estep et al, 1984); the central North Pacific Ocean (Hoepffner and Haas, 1990); Norwegian coastal waters (Eikrem and Throndsen, 1993; 1998); a Canadian fjord (Smith and Hobson, 1994); New Zealand coastal waters (Rhodes and Burke, 1996); and the Mediterranean Sea (Eikrem and Throndsen, 1998).

Toxicity.

A bloom of *C. leadbeateri* was reportedly responsible for a massive fish kill (600 tonnes of farmed Atlantic salmon) in Norway in 1991 (Aune et al, 1992; Eikrem and Throndsen, 1993). Water samples from the bloom were toxic to *Artemia* nauplii and to nerve cell preparations; however, cultures of *C. leadbeateri* established from the bloom were non-toxic to *Artemia* nauplii, nerve cell preparations and erythrocytes (Edvardsen, 1993; Meldahl et al, 1994, 1995; Simonsen and Moestrup, 1997). Cultures grown under varying phosphate concentrations and fed to *Artemia* nauplii also failed to demonstrate toxicity (Edvardsen, 1993).

This species was not cultured in the present work, and hence was unable to be tested for toxicity.

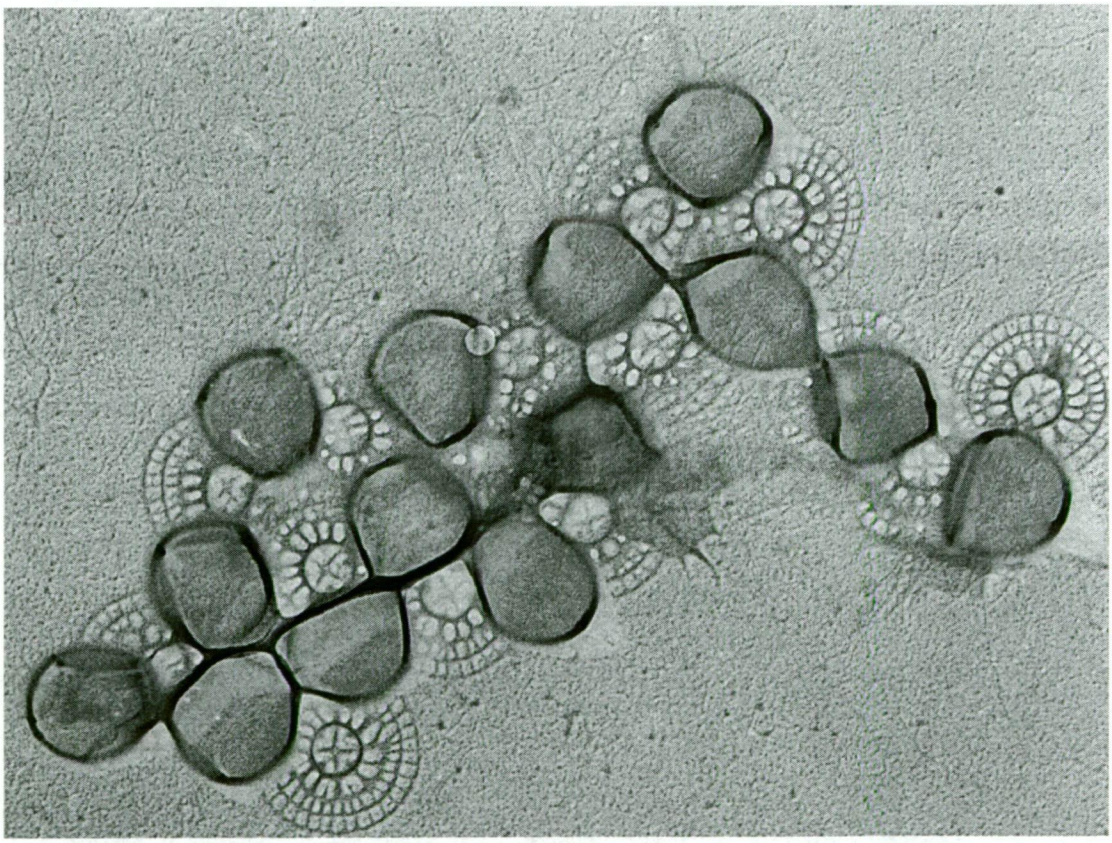


Fig. 4.36: *C. leadbeateri* scales (0.3 and 0.35 μm); from Pipeclay Lagoon.

(Micrograph no: 5444)

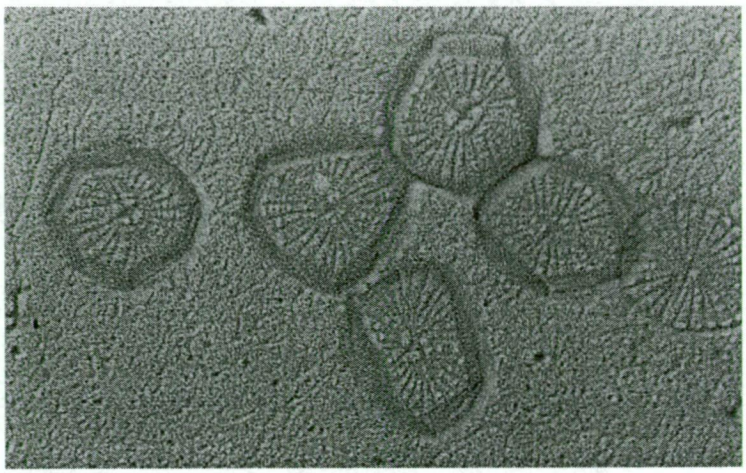


Fig. 4.37: *C.* aff. *leadbeateri* scales (0.25 μm) with angular upright rims; from Pipeclay Lagoon

(Micrograph no: 5453)

Table 4.18: Size and structure of *Chrysochromulina leadbeateri* rimless scales from different locations.

SOURCE	DIMENSIONS (μm)	NO. OF CONCENTRIC RINGS	NO. OF PORES IN OUTER BAND	NO. OF PORES IN MIDDLE BAND	NO. OF PORES IN INNER BAND
N. Norway (Eikrem & Throndsen, 1998)	0.25 - 0.35	4	25 - 30	24 - 28	16
W. Norway (Leadbeater, 1972)	0.35	4	30 - 32	28	14 - 16
S. Norway (Eikrem & Throndsen, 1998)	0.3 (n=3)	4	30 - 32	24	16
Mediterranean (Eikrem & Throndsen, 1998)	0.47 (n=1)	4	28 - 30	28 - 30	16
Australia (Hallegraeff, 1983)	0.20 - 0.25	4	25	25	25
North Atlantic (Estep et al, 1984)	0.3	3	24 - 28	-	ND
North Pacific (Hoepffner & Haas, 1990)	0.2 - 0.3	3	24 - 28	-	ND
Tasmania, Australia - Pipeclay Lagoon & Southport	0.35 (n=2)	4	30 - 32	34 - 36	13 - 15
Tasmania, Australia - Pipeclay Lagoon	0.23 x 0.20 (n=1)	Numerous	28	-	-

ND= not determined

Table 4.19: Size and structure of *Chrysochromulina leadbeateri* rimmed scales from different locations.

SOURCE	DIMENSIONS (μm)	RIM HEIGHT (μm)	CENTRAL CROSS	OUTER BAND (No. of perforations)	INNER BAND (No. of perforations)	RADIATING RIDGES (No.)	CONCENTRIC RINGS	PATTERNLESS SURFACE
N. Norway (Eikrem & Throndsen, 1998)	0.3 - 0.4	0.1 - 0.13	Yes	Yes (25 - 30)	No	No	2	No
W. Norway (Leadbeater, 1972b)	0.35	ND	Yes	No	Yes (12 - 14)	No	1	No
S. Norway (Eikrem & Throndsen, 1998)	0.29 x 0. 24 (n=1)	c. 0.03 (n=1)	Yes	Yes (c. 24)	Yes (12)	Yes	3	No
Mediterranean (Eikrem & Throndsen, 1998)	0.3 - 0.4 x 0.3 ($\bar{x}$ =0.4 x 0.3; n=5)	ND	No	No	No	No	0	Yes
Australia (Hallegraeff, 1983)	0.2 - 0.25	ND	Yes	Yes (25)	No	Yes (25)	2	No
North Atlantic (Estep et al, 1984)	0.4	ND	Yes	No	No	No	1	No
North Pacific (Hoepffner & Haas, 1990)	0.2 - 0.3	c. 0.05 (n=1)	Yes	No	No	No	1	No
Tasmania, Australia - Pipeclay & Southport	0.25 - 0.35	ND	No	No	No	No	0	Yes
Tasmania, Australia - Pipeclay Lagoon	0.2 - 0.3 x 0.20 ($\bar{x}$ =0.2; n=5)	c. 0.04 (n=5)	Yes	No	No	Yes (22 - 28)	Numerous	No

Chrysochromulina mactra **Manton** **Figs. 4.38 - 4.40**

Micrographs: Manton, 1972; Figs. 11 - 15.

Present Findings.
Scales were found in the Pirates Bay sample.

This is a new record for Australian waters.

Description.
Chrysochromulina mactra has two types of scales: characteristic "tub-shaped" scales and oval plate scales.

"Tub-shaped" scales had straight, slightly diverging, sides and a flat oval base (Fig. 4.40). The base was patterned with numerous radiating ridges, arranged in quadrants and extending to the scale edge, and had a cruciform centre. The sides of the scales were very thin and transversely striated. Scales were approximately 1.5 µm high, with base dimensions of 1.3 x 0.8 µm (n=2).

Plate scales were patterned with radiating ridges in quadrants, extending to the rim on one surface, and to a broad peripheral band, c. 0.20 - 0.25 µm wide ($\bar{x}$=0.24; n=3), on the other surface (Figs. 4.38, 4.39). Scales had a cruciform centre and the peripheral band had fine concentric fibrils. Two sizes of plate scales were found: larger scales were 2.0 - 2.1 x 1.6 - 1.7 µm ($\bar{x}$=2.02 x 1.62 µm; n=4) while smaller scales were 1.3 x 0.9 - 1.0 µm ($\bar{x}$=1.29 x 0.90 µm; n=3), approximately half the size of the larger scales.

Both "tub-shaped" and plate scales closely matched those of the type species (Manton, 1972), although in the type description, the size of the smaller plate scales was not given. This was found to be 1.2 x 0.8 µm (Manton, 1972; Fig. 14), agreeing with sizes measured in the Tasmanian material.

Distribution.
C. mactra has been recorded from the UK, Denmark, Norway, Yugoslavia, Algiers and New Zealand (Moestrup, 1979, and references therein).

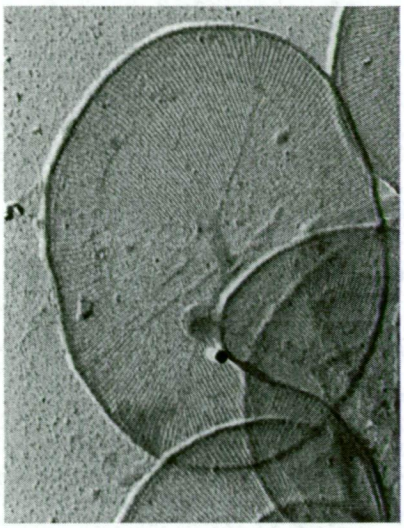


Fig. 4.38: *C. mactra* large plate scale (2.0 x 1.5 μm); from Pirates Bay

(Micrograph no: 5104)

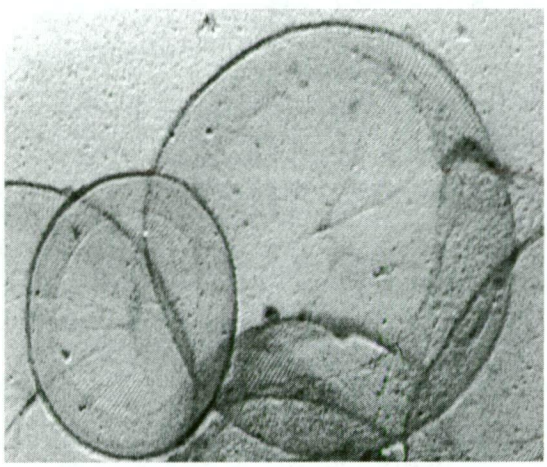


Fig. 4.39: *C. mactra* large plate scales (1.8 x 1.6 μm) and small plate scales (1.3 x 1.0 μm) showing broad peripheral band; from Pirates Bay

(Micrograph no: 5104)

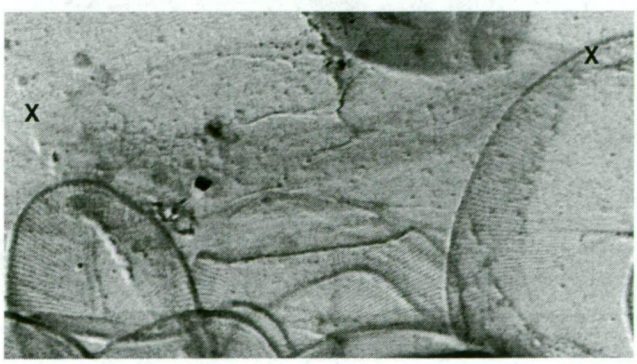


Fig. 4.40: *C. mactra* "tub-shaped" scale (c. 1.5 μm high) with edges of scales marked (X); from Pirates Bay

(Micrograph no: 5104)

***Chrysochromulina mantoniae* Leadbeater** **Fig. 4.41**

Micrographs: Leadbeater, 1972a; Fig. 36 - 39.
Manton and Leadbeater, 1974; Figs. 43 - 48.
Manton, 1982; Figs. 8 - 9.
Beech, 1983; Plate 2.4B.
Thomsen et al, 1994; Fig. 12.

Present Findings.
Scales were found in the Port Huon sample.

Description.
Two types of oval plate scales were recorded (Fig. 4.41). The smaller scale was 0.5 x 0.4 µm ($\bar{x}$=0.51 x 0.38 µm; n=5) with a conspicuous inflexed rim, 0.07 - 0.11 µm wide ($\bar{x}$=0.08; n=5). One surface appeared plain, while the other surface had c. 36 radiating ridges, arranged in quadrants, and extending from a central patternless area to the scale edge. Underlying these ridges, faint concentric rings were seen.

The larger scale was 1.4 - 1.5 x 1.0 µm ($\bar{x}$=1.42 x 0.96; n=6), with c. 120 slightly curved radiating ridges arranged in quadrants, and a distinct raised rim. This pattern was more clearly seen on one surface .

These two scale types closely matched those of the type species (Leadbeater, 1972a). In fact, *C. mantoniae* scales have very similar size and structure, regardless of geographical location (Table 4.20).

C. mantoniae also has a small number of spine scales, usually found at each end of the cell (Leadbeater, 1972a), but these were not seen in the Tasmanian material.

Distribution.
C. mantoniae has been recorded from Norway, Denmark, Yugoslavia, Algeria, the Galapagos Islands, South Africa, and Australia (Leadbeater, 1972a; Leadbeater, 1974; Manton and Leadbeater, 1974; Manton, 1982; Beech, 1983).

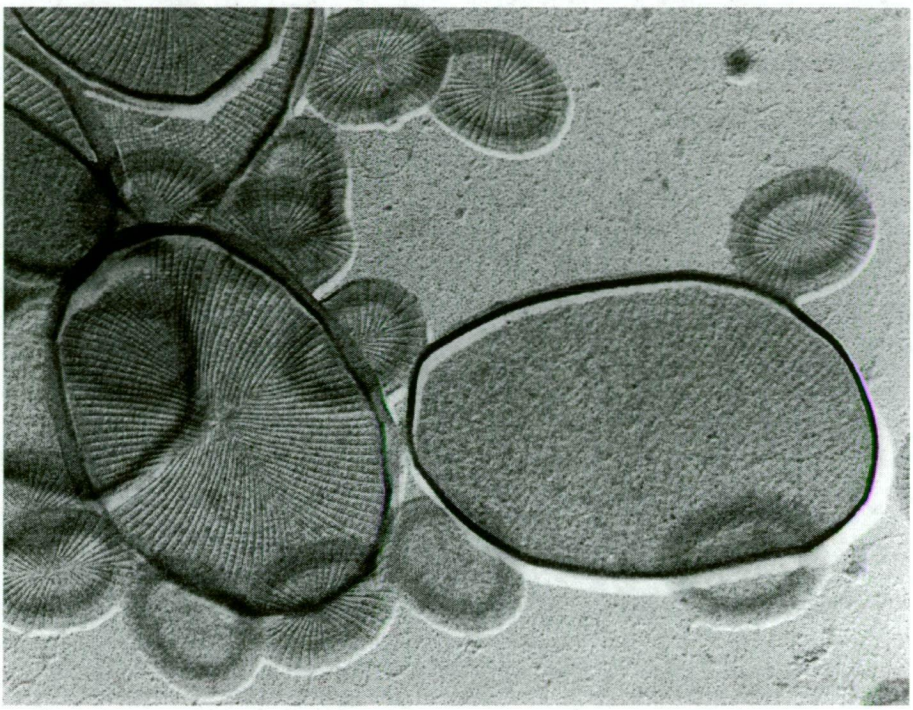


Fig. 4.41: *C. mantoniae* large plate scales (1.4 x 1.0 μm) and small plate scales (0.5 x 0.4 μm) - distal and proximal views; from Port Huon

(Micrograph no: 5301)

Table 4.20: *C.hrysochromulina mantoniae* plate scales from different locations.

SOURCE	SMALL SCALES			LARGE SCALES	
	Dimensions (μm)	*Rim Width (μm)*	*No. of Radiating Ridges*	*Dimensions (μm)*	*No. of Radiating Ridges*
Norway *(type)* (Leadbeater, 1972)	0.5 x 0.4	0.1	ND	1.5 x 1.1	ND
South Africa (Manton, 1982)	0.5 x 0.3 - 0.4 ($\bar{x}$ =0.5 x 0.4; n=5)	0.07 - 0.1 ($\bar{x}$ =0.08; n=5)	36	1.5 - 1.6 x 1.0 ($\bar{x}$ =1.54 x 0.98; n=3)	c. 120
Victoria, Australia (Beech, 1983)	0.4 - 0.5 x 0.3 - 0.4 ($\bar{x}$ =0.46 x 0.37, n=4)	0.07 (n=3)	36	1.4 x 1.3 (n=1)	c. 100
Tasmania, Australia	0.5 x 0.4 ($\bar{x}$ =0.51 x 0.38; n=5)	0.07 - 0.11 ($\bar{x}$ =0.08; n=5)	36	1.4 - 1.5 x 1.0 ($\bar{x}$ =1.42 x 0.96; n=6)	c. 120

ND = Not determined

***Chrysochromulina minor* Parke et Manton** **Figs. 4.42 - 4.45**

Micrographs: Parket et al, 1955; Figs. 62 - 64.
Hallegraeff, 1983; Fig. 10.

Present Findings.

Scales were found in samples from Pipeclay Lagoon and Coles Bay.

Description.

Scales were thin and finely sculptured as seen in Fig. 4.42. Two types of scales were found: large hexagonal to octagonal scales (Fig. 4.43), and smaller circular to oval scales (Fig. 4.45) . The larger scales were 0.8 x 0.5 µm (n=4), and the smaller scales were 0.35 - 0.4 µm ($\bar{x}$=0.38 µm; n=6). Both were patterned with fine radiating ridges arranged in quadrants (c. 20 ridges per quadrant in the smaller scales and c. 30 ridges per quadrant in the larger scales). On one side of the smaller scales, a broad peripheral band composed of concentric rings was seen (Fig. 4.45). The larger scales had an upright rim. Both scale types closely matched those illustrated for the type species (Parke et al, 1955).

Distribution.

C. minor was orginally reported from the UK, with other records from Norway, Australia and Canada (Leadbeater, 1972b; Hallegraeff, 1983; Smith and Hobson, 1994).

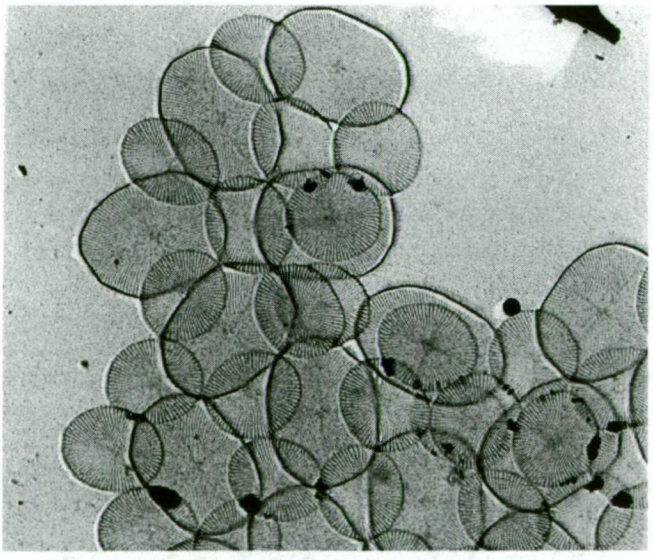


Fig. 4.42: *C. minor* finely sculptured scales (0.8 x 0.5 µm and 0.35 - 0.40 µm); from Pipeclay Lagoon

(Micrograph no: 5022)

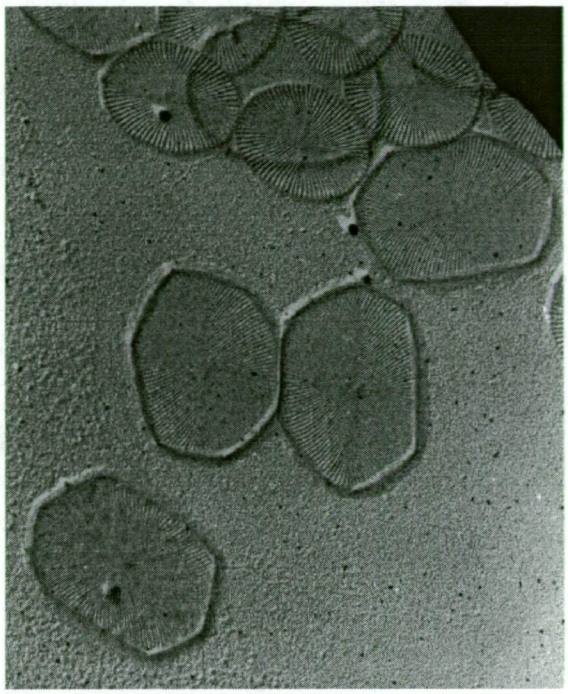


Fig. 4.43: *C. minor* hexagonal and octagonal plate scales (0.8 x 0.5 μm); from Coles Bay

(Micrograph no: 5513)

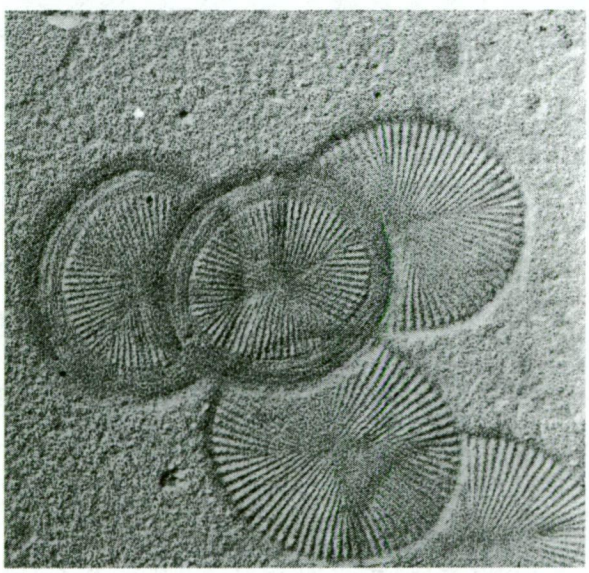


Fig. 4.45: *C. minor* small plate scales (0.35 μm) showing broad peripheral band; from Coles Bay

(Micrograph no: 5513)

***Chrysochromulina novae-zelandiae* Moestrup** **Fig. 4.46**

Micrographs: Moestrup, 1979; Figs. 13 - 18.
Hallegraeff, 1983; Fig. 11.

Present Findings.
A group of scales was found in the Fleurty Point sample. Scales were also found in a Dru Point sample.

Description.
Chrysochromulina novae-zelandiae has three types of scales (Moestrup, 1979), only one of which was observed (Fig. 4.46) in this survey. These oval "under-layer" scales were 0.4 - 0.5 x 0.3 µm in size ($\bar{x}$=0.45 x 0.29; n=5) and had distinctive patternless rims, ranging from 0.04 - 0.08 µm wide ($\bar{x}$=0.05 µm; n=5). Scales had a characteristic pattern of radiating ridges (c. 50), arranged in quadrants, which extended from a central cruciform area to the scale rim.

These scales matched those described in previous reports (Moestrup, 1979; Hallegraeff, 1983). However, neither the smaller circular scales nor the triangular scales, described by Moestrup as characteristic of this species, were seen. These scale types have not been recorded since the original description.

Distribution.
Initially recorded from New Zealand (Moestrup, 1979) and then from south-east Australia (Hallegraeff, 1983), *C. novae-zelandiae* has not yet been reported from the northern hemisphere.

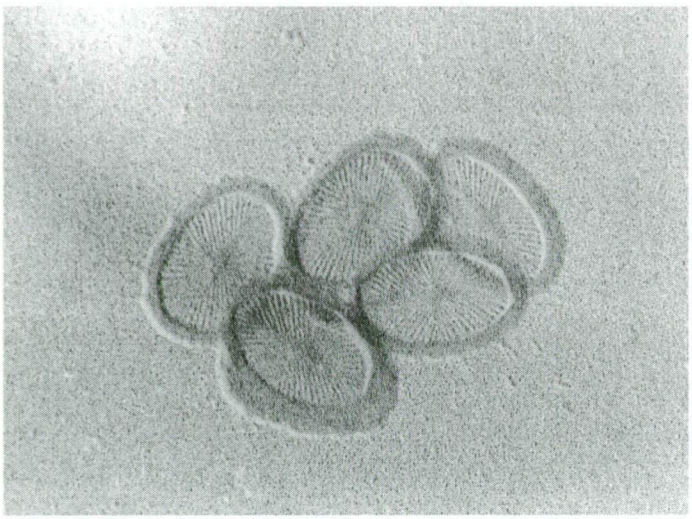


Fig. 4.46: *C. novae-zelandiae* scales (0.5 x 0.3 μm); from Fleurty Point

(Micrograph no: 4.46)

***Chrysochromulina pachycylindra* Manton et Oates** **Figs. 4.47 - 4.48**

Micrographs: Manton, Oates and Course, 1981; Figs. 1 - 5.
Beech, 1983; Plate 2.4 C - E.
Hoepffner and Haas, 1990; Fig. 11.
Rhodes and Burke, 1996; Fig. 6A.

Present Findings.
Scales were found in samples from the Derwent River, Dru Point, Pipeclay Lagoon, Eaglehawk Neck and Honey Moon Bay.

Scales were also observed in enrichment cultures derived from Dru Pt (GSe/10 (+GeO_2) medium), Coles Bay (ML medium), Honey Moon Bay (GSe medium) and Storm Bay (ML medium).

Description.
Two scale types characterise this species: long cylindrical spine scales and plate scales with distinct rims (Manton et al, 1981).

Cylindrical spine scales ranged from 5 - 7 µm in length ($\bar{x}$=5.7 µm; n=6), 0.3 - 0.4 µm in width ($\bar{x}$=0.32 µm; n=6), and had a base diameter of 0.8 - 1.0 µm (Fig. 4.47). They had thin straight sides, which widened slightly at the base, and a flat rimmed base plate.

Plate scales were usually circular, 0.6 - 1.0 µm diameter ($\bar{x}$=0.77; n=13), and patterned with numerous radiating ridges, arranged in quadrants and extending to the scale rim. They had an obvious central cruciform thickening and a raised perforated rim (Fig. 4.48).

One smaller elliptical plate scale (0.68 x 0.53 µm) with similar patterning was also seen in an enrichment culture derived from a Coles Bay sample. These smaller plate scales have also been recorded by Manton et al, 1981 (Fig. 3A) and Rhodes and Burke, 1996 (Fig. 6).

There is considerable variation in *C. pachycylindra* scale size, as reported by several authors (Table 4.21). Cylindrical spine scale length ranged from 3 - 18 µm, and plate scale diameter ranged from 0.6 - 2.2 µm. Scale structure appears to be fairly constant, with only minor variations. For example, Beech (1983) reported plate scales of "thinner texture and with more compressed radiating ridges" from south-

east Australia, and Manton et al (1981) noted faint spiral threads overlying radiating ridges on the distal surface of a plate scale from the Galapagos Islands.

The cylindrical spine scales of *C. pachycylindra* may be confused with those of *C. quadrikonta.* However, *C. quadrikonta* spine scales are generally shorter and have a wider base diameter (Rhodes et al, 1994, Fig. 3B; Rhodes and Burke, 1996, Fig. 3B).

Distribution.

C. pachycylindra was orginally described from South Africa, the English Channel and the Galapagos Islands, at temperatures ranging from 10 - 23°C (Manton et al, 1981). It has since been reported from the North Atlantic and North Pacific Oceans (Estep et al, 1984; Hoepffner and Haas, 1990), and from south-east Australia and New Zealand (Beech, 1983; Rhodes and Burke, 1996).

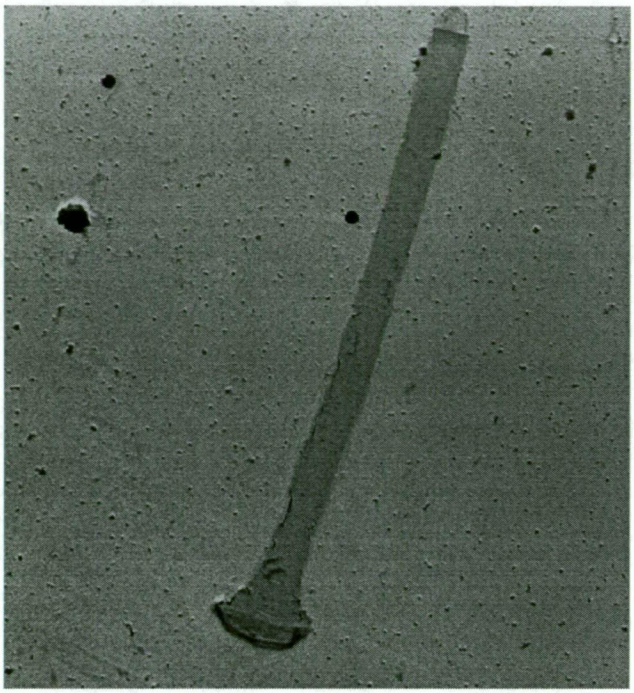


Fig. 4.47: *C. pachycylindra* spine scale (7 μm long); from a Storm Bay enrichment culture

(Micrograph no: 5571)

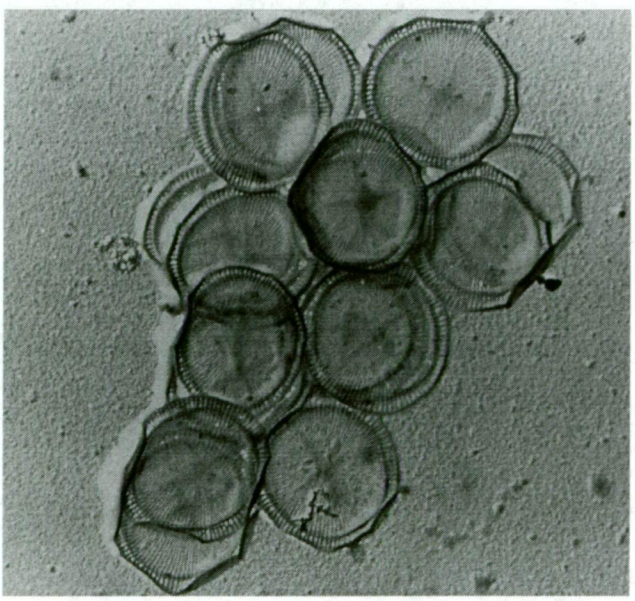


Fig. 4.48: *C. pachycylindra* plate scales (c. 0.7 μm diam.); from Eaglehawk Neck

(Micrograph no: 5418)

Table 4.21: ***Chrysochromulina pachycylindra*** **scales from different locations.**

SOURCE	CYLINDRICAL SPINE SCALES		PLATE SCALES
	Height (µm)	*Width (µm)*	*Diameter (µm)*
Galapagos Islands *(type)* (Manton et al, 1981)	≤ 16	0.5 - 0.7	0.6 - 1.0
South Africa (Manton et al, 1981)	6	0.3 - 0.4 (n=2)	0.6 - 1.0
Australia (Beech, 1983)	6 (n=1)	0.6 (n=1)	1.4 - 2.2 (n=3)
North Atlantic Ocean (Estep et al, 1984)	18	ND	0.7
North Pacific Ocean (Hoepffner & Haas, 1990)	8	0.6 (n=1)	0.65
New Zealand (Rhodes & Burke, 1996)	3 (n=1)	0.3 (n=1)	0.8 - 1.2 (n=2)
Tasmania, Australia	5 - 7 ($\bar{x}$ =5.7; n=6)	0.3 - 0.4 ($\bar{x}$ =0.32; n=6)	0.6 - 1.0 ($\bar{x}$ =0.77; n=13)

ND = Not determined

***Chrysochromulina parkae* Green et Leadbeater** **Figs. 4.49 - 4.53**

Micrographs: Green and Leadbeater, 1972; Plates 1 - 4.
Beech 1983; Plate 2.6.
Hallegraeff, 1983; Fig. 12.

Present Findings.
Whole cells were recorded from the Derwent River. Scales were found in samples from the Derwent River, Dru Point, Southport, Pipeclay Lagoon, Eaglehawk Neck and Honey Moon Bay.

Chrysochromulina parkae grew in GSe (+GeO_2) enrichment cultures derived from Derwent River samples. Scales were also found in enrichment cultures from Pirates Bay (K/10 medium) and Honey Moon Bay (GSe medium) .

Description.
Cell size was approximately 10 x 5 µm. Although cells lost flagella and haptonema during sample preparation, the scale covering was usually retained (Fig. 4.49).
C. parkae has four scale types, all of which were seen in this material, namely plate scales of three different sizes and long "spoon-like" spine scales found at one end of the cell (Green and Leadbeater, 1972).

The largest plate scales were elliptical to diamond-shaped (1.8 - 2.6 x 1.1 - 1.6 µm; $\bar{x}$=2.3 x 1.4 µm; n=22), with a broad raised rim. They were patterned with crescent-shaped and radiating fibrils on both surfaces, the distal surface having a more irregular pattern of fibrils (Fig. 4.52).

Medium-sized plate scales were oval (0.8 - 2.2 x 0.6 - 1.4 µm; $\bar{x}$=1.6 x 1.0 µm; n=57), with a marginal pattern of small pores and ridges. In addition, a faint peripheral band of concentric fibrils was present on the distal surface. Scale surfaces were patterned with fine radiating ridges and crescent-shaped fibrils (Fig. 4.51).

Small plate scales were elliptical (1.0 - 1.5 x 0.3 - 0.4 µm; $\bar{x}$=1.3 x 0.3 µm; n=3), with a peripheral band of concentric fibrils. This band was narrower than that of the medium-sized plate scales. Scales had an irregular surface pattern of crescent-shaped and radiating fibrils, similar to that of the larger plate scales (Fig. 4.52). Fewer of these scales were seen in comparison to the other scale types.

Spine scales had a long tapering spine, usually 6 - 8 μm length ($\bar{x}$=7 μm; n=4), although one spine scale was found with a spine length of 17 μm (Fig. 4.50) in the Southport sample. Scales had "spoon-like" base, patterned with crescent-shaped and radiating fibrils (Fig. 4.50). Green and Leadbeater (1972) observed regular cross-banding along the length of the spine, but this was not apparent in the Tasmanian material.

Scale sizes from wild and cultured material were similar, and were generally smaller than those of the type material (Green and Leadbeater, 1972). There was considerable variation in scale sizes reported for this species, particularly for spine scale length (Table 4.22). Spine scales from Australian waters were usually shorter than other material.

Scale morphology also varied. For example, Green and Leadbeater (1972) found that the peripheral band in medium-sized plate scales was considerably wider in material from the UK in comparison to Norwegian specimens. Beech (1983) reported large, distinctly diamond-shaped, plate scales with significantly wider rims from Australian samples, similar to the one shown in Fig. 4.53.

Distribution.

C. parkae has been reported from coastal and off-shore waters in both northern and southern hemispheres, including those of: Norway, UK (Green and Leadbeater, 1972), Denmark (Leadbeater, 1972b; Manton and Leadbeater, 1974), Yugoslavia, Algeria (Leadbeater, 1974), Canada, (Smith and Hobson, 1994), the North Atlantic Ocean (Estep et al, 1984), New Zealand (Moestrup, 1979) and Australia (Beech, 1983; Hallegraeff, 1983).

Fig. 4.49: *C. parkae* cell (10 x 5 μm) with scales; from a Derwent River enrichment culture

(Micrograph no: 5318)

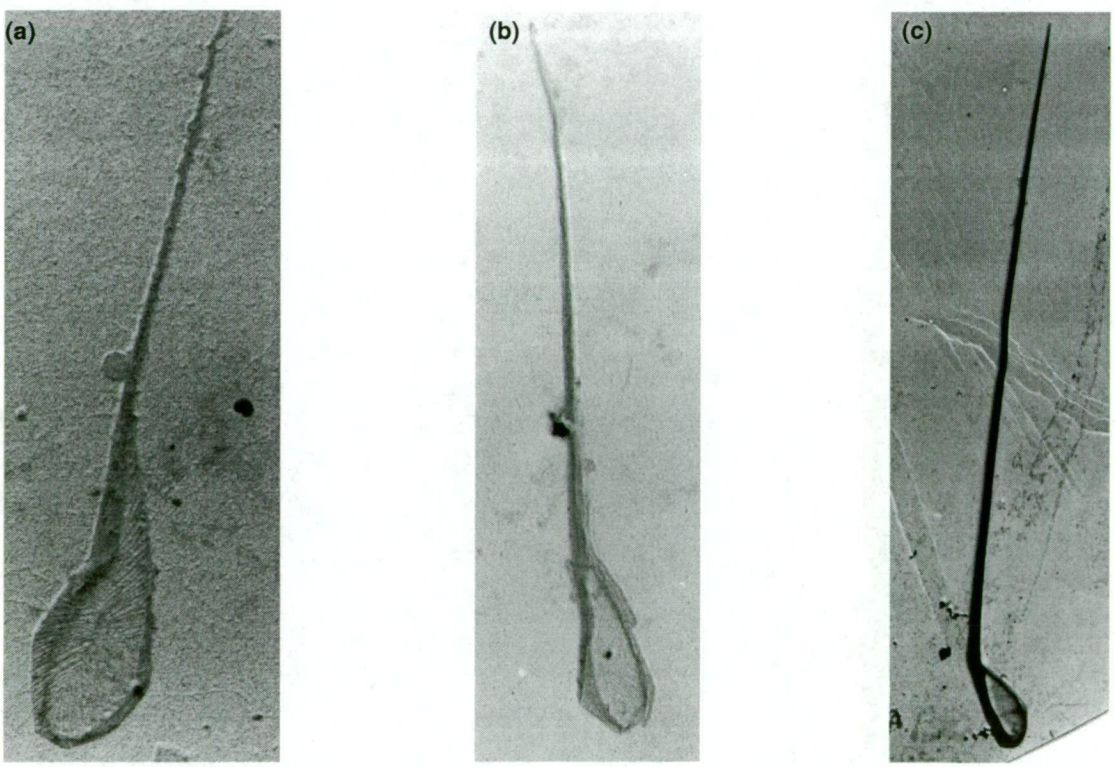


Fig. 4.50: *C. parkae* "spoon" scales: (a), (b) from a Derwent enrichment culture (6 - 8 μm long); (c) from Southport (17 μm long)

(Micrograph no: 5314, 5319, 5405)

Fig. 4.51: *C. parkae* medium-sized plate scales (1.5 x 1.0 μm); from Honey Moon Bay

(Micrograph no: 5350)

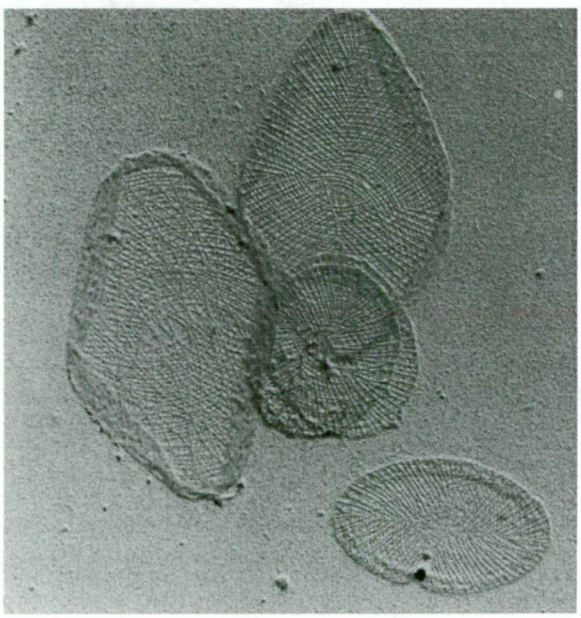


Fig. 4.52: *C. parkae* large, diamond-shaped scales (2.0 x 1.5 μm), and small elliptical plate scales (1.0 x 0.4 μm); from the Derwent River

(Micrograph no: 5346)

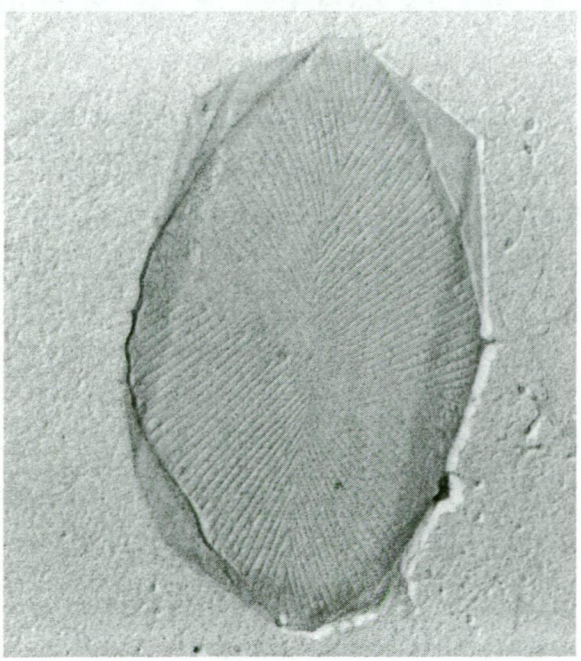


Fig. 4.53: *C.* aff. *parkae* large plate scale (2.5 x 1.5 μm); from a Derwent enrichment culture

(Micrograph no: 5322)

Table 4.22: Scale sizes of *Chrysochromulina parkae* from different locations.

SOURCE	SMALL SCALES		MEDIUM SCALES		LARGE SCALES		SPINES
	Length (µm)	*Width (µm)*	*Length (µm)*	*Width (µm)*	*Length (µm)*	*Width (µm)*	*Length (µm)*
Norway, UK (Green & Leadbeater, 1976)	1.3 - 2.8	0.6 - 1.2	1.3 - 4.1	0.7 - 2.8	2.2 - 5.0	1.3 - 3.5	20 - 31
New Zealand (Moestrup, 1979)	-	-	1.8 - 2.1	1.0 - 1.6	2.2 - 2.8	1.6 - 1.7	-
Victoria, Australia (Beech, 1983)	0.9 (n=1)	0.5 (n=1)	1.1 - 1.4 ($\bar{x}$ =1.3; n=6)	0.6 - 0.8 ($\bar{x}$ =0.8; n=6)	2.0 - 2.8 ($\bar{x}$ =2.5; n=5)	1.2 - 1.6 ($\bar{x}$ =1.5; n=5)	5 - 12 ($\bar{x}$ =8.2; n=6)
East Australian Current (Hallegraeff,1983)	1.9	1.3	3.4	2.4	5.2	3.4	17
North Atlantic Ocean (Estep et al, 1984)	1.5	0.7	-	-	2.2	1.2	16 - 31
Tasmania, Australia	1.0 - 1.5 ($\bar{x}$ =1.3; n=3)	0.3 - 0.4 ($\bar{x}$ =0.3; n=3)	0.8 - 2.2 ($\bar{x}$ =1.6; n=57)	0.6 - 1.4 ($\bar{x}$ =1.0; n=57)	1.8 - 2.6 ($\bar{x}$ =2.3; n=22)	1.1 - 1.6 ($\bar{x}$ =1.4; n=22)	6 - 8; 17 ($\bar{x}$ =7; n=4)

Chrysochromulina aff. *polylepis* Manton et Parke — Figs. 4.54 - 4.55

Micrographs: Manton and Parke, 1962; Plates 2 - 4.
Dahl et al, 1989; Fig. 3B - C.
Paasche et al, 1990; Figs. 2 - 8.
Edvardsen and Paasche, 1992; Fig. 1.
Eikrem and Throndsen, 1993; Figs. 4 - 6.
Throndsen et al, 1995; Figs. 13 - 16.
Edvardsen et al, 1996; Figs. 1 - 5.
Edvardsen and Medlin, 1998; Figs. 3 - 4.

Present Findings.
Scales were found in one sample from Honey Moon Bay.

Description.
Three scale types were found, which generally had similarities to those of *Chrysochromulina polylepis.*

Small oval scales were 0.6 - 0.7 x 0.4 - 0.5 µm in size ($\bar{x}$ = 0.7 x 0.5 µm; n=18) and were the most numerous scale type. They had a perforated pattern, formed by c. 40 radiating ridges and c. 5 concentric rings, and a narrow patternless rim on one surface (Fig. 4.54). The number of perforations increased from 2 - 4 circular perforations at the scale centre, to 46 - 48 elongate perforations around the scale rim. Two raised points separated the central perforations. This scale type matched that given in the original description of *C. polylepis* (Manton and Parke, 1962; Figs. 5, 7, 8 and 9) and has since been recorded by various authors (Dahl et al, 1989, Fig. 3C; Paasche et al, 1990, Figs. 2 and 7; Edvardsen and Paasche, 1992, Fig. 1b; Eikrem and Throndsen, 1993, Fig. 4; Throndsen et al, 1995, Fig. 14; Edvardsen and Medlin, 1998, Fig. 4).

Elliptical scales were 1.0 - 1.1 x 0.4 - 0.5 µm in size ($\bar{x}$ =1.0 x 0.5 µm; n=4). They had a dominant pattern of numerous straight radiating ridges arranged in quadrants, and a distinct outer rim, c. 0.05 µm wide. Radiating ridges extended from a cruciform centre right to the scale edge, and crossed elliptical fibres to give a faint meshwork pattern (Fig. 4.54). These scales closely matched the type description given by Manton and Parke based on samples from the Irish Sea (1962; Figs. 6 and 10), and were also recorded from Norwegian waters by Paasche et al, (1990; Fig. 2) and Edvardsen and Medlin (1998; Fig. 4).

However, the scales lacked the distinct perforations seen in other Scandinavian material described as *C. polylepis* (Eikrem and Throndsen, 1993, Fig. 4; Moestrup, 1995, Fig. 16.6d; Throndsen et al, 1995, Fig. 16).

The third type of scale was scarce and, of the three scale types, showed the most variation when compared with the type material. This large oval scale (1.2 x 0.7 μm; n=1) had a dominant pattern of slightly curved radiating ridges arranged in quadrants, a central cross, and a patternless raised rim, c. 0.07 μm wide (Fig. 4.55). Apart from an outer ring of c. 80 elongate perforations, the scale lacked the overall meshwork pattern shown in the type material (Manton and Parke, 1962; Figs. 5, 7, 9 and 11) and subsequently illustrated by other authors (Dahl et al, 1989, Fig. 3C; Paasche et al, 1990, Figs. 2 and 7; Edvardsen and Paasche, 1992, Fig. 1b; Eikrem and Throndsen, 1993, Fig. 4; Throndsen et al, 1995, Fig. 13; Moestrup and Thomsen, 1995, Fig. 16.6b; Edvardsen and Medlin, 1998, Fig. 4).

Spined scales of *C. polylepis* are uncommon and were not found in the Tasmanian material.

C. polylepis has been reported to have an aberrant form, which has only three scale types (Paasche et al, 1990; Figs. 3 - 6, 8), and is possibly a life cycle stage (Edvardsen and Paasche, 1992; Edvardsen et al, 1996). One scale type has a central spine supported by four decurrent ridges and the other two oval scale types are similar, but larger, than those of the type species. No scales of the aberrant form were found in this survey.

Given the variation in scale size and morphology (Table 4.23), as well as recently described genetic differences between *C. polylepis* strains (Edvardsen and Medlin, 1998), the taxonomy of this species urgently requires revision.

Distribution.

C. polylepis was originally described from the Irish Sea (Manton and Parke, 1962) and is a well-known species from Scandinavian coastal waters (Edvardsen and Paaschae, 1998, and references therein). It has also been reported from Canada (Taylor, 1993) and Australia (Moestrup and Thomsen, 1995), although no micrographs have been published to confirm species identification.
C. cf. *polylepis* has been recorded from New Zealand (Rhodes and Burke, 1996).

Toxicity.

In 1988, an extensive bloom of *C. polylepis* caused the death of 900 tonnes of farmed salmonid fish, as well as affecting a wide range of naturally occurring marine organisms including invertebrates, macroalgae, plankton and bacteria (Dahl et al, 1989; Torbjørn and Lømsland, 1990; Nielsen et al, 1990). This was the first incidence of a toxic *Chrysochromulina* bloom in marine waters and resulted in numerous investigations on all aspects of both the bloom-causing species and the bloom itself.

C. polylepis was originally described as non-toxic to fish (Manton and Parke, 1962), an observation supported by Dahl et al (1989) who also found no appreciable toxicity to a range of test organisms. However, Jebram (cited in Moestrup, 1994) reported that, while young cultures of *C. polylepis* were an excellent food for the bryozoan *Electra pilosa*, old cultures fed in high concentrations were toxic. Studies have shown cultures of both the authentic and aberrant forms of *C. polylepis* to be toxic to *Artemia* nauplii, with cultures grown under phosphorous limitation having a greater toxic effect (Edvardsen and Paasche, 1992; Edvardsen, 1993; Rhodes et al, 1994; Edvardsen et al, 1996; Simonsen and Moestrup, 1997). Consequently, it is likely that the toxicity of *C. polylepis* depends on growth stage and environmental conditions.

Extracts of *C. polylepis* cultures have also been found to be toxic to fish, mouse, human and horse erythocytes (Edvardsen et al, 1990; Yasumoto et al, 1990; Meldahl et al, 1995; Simonsen and Moestrup, 1997), and to nerve cell preparations (Meldahl et al, 1994, 1995).

In the present work, *C. polylepis* did not grow in enrichment culture, and hence was unable to be tested for toxicity.

Fig. 4.54: *C.* aff. *polylepis* small oval scales (0.7 x 0.5 μm) and larger elliptical scales (1.0 x 0.5 μm); from Honey Moon Bay

(Micrograph no: 5365)

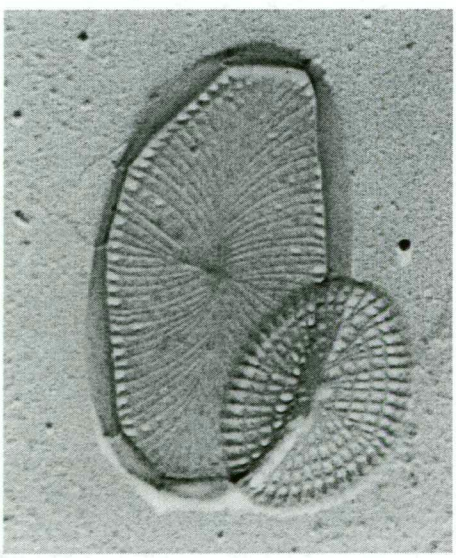


Fig. 4.55: *C.* aff. *polylepis* large oval scale (1.2 x 0.7 μm) with radiating ridges, and small oval scale (0.6 x 0.5 μm) with perforations; from Honey Moon Bay

(Micrograph no: 5374)

Table 4.23: Variation in scales of *Chrysochromulina polylepis* and *C.* aff.*polylepis* from different locations.

SOURCE	SMALL OVAL SCALES		LARGE OVAL SCALES		ELLIPTICAL SCALES		SPINED SCALES		
	Size (µm)	*Perforated pattern*	*Size (µm)*	*Perforated pattern*	*Size (µm)*	*Perforated pattern*	*Size (µm)*	*Perforated pattern*	*Forked projection*
C. polylepis									
UK (Manton & Parke, 1962)	0.8 x 0.6	Yes	1.4 x 1.2	Yes	1.2 x 0.6	No	2.5 x 0.9	No	Yes
Norway									
(Dahl et al, 1989)	0.7 x 0.5 (n=1)	Yes	1.2 x 0.7 (n=1)	Yes	1.0 x 0.5 (n=1)	Yes	c. 2.0 x 0.5 (n=1)	No	Yes (>1 branching)
(Paasche et al, 1990)	0.7	Yes	1.4	Yes	1.2	No	2.5	Yes	Yes
(Edvardsen & Paasche, 1992)	0.7 - 0.8 x 0.5 - 0.6 (n=5)	Yes	1.3 - 1.4 x 0.9 (n=2)	Yes	-	-	2.5 x 0.6 (n=1)	No	Yes
(Eikrem & Throndsen, 1993)	0.8 x 0.5 (n=2)	Yes	1.4 x 0.9 (n=1)	Yes	1.1 - 1.2 x 0.5 (n=4)	Yes	5.5 x 1.5 n=1	Yes	Yes (at both ends)
(Edvardsen et al, 1996)	0.7 x 0.5 (n=3)	Yes	1.2 x 0.8 (n=1)	Yes (very faint)	1.1 x 0.6 (n=1)	No	2.2 x 0.9 n=1	No	Yes
Denmark									
(Throndsen et al, 1995)	0.7 - 0.8 x 0.5 - 0.6	Yes	1.3 - 1.4 x c. 0.8	Yes	c. 1.0 x 0.5	Yes	c. 2.5 x 0.5	Yes	Yes
C. aff. polylepis									
New Zealand (Rhodes & Burke, 1996)	-	-	1.2 - 1.3 (n=1)	Yes	-	-	-	-	-
UK (Edvardsen & Medlin, 1998)	0.5 x 0.4 (n=3)	No	0.9 (n=1)	No	0.8 - 0.9 x 0.5 (n=4)	No	1.5 x 0.75 (n=1)	No	Yes (at both ends)
Tasmania, Australia	0.6 - 0.7 x 0.4 - 0.5 ($\bar{x}$ = 0.7 x 0.5; n=18)	Yes	1.2 x 0.8 (n=1)	No	1.0 - 1.1 x 0.4 - 0.5 ($\bar{x}$ =1.0 x 0.5; n=4)	Yes (very faint)	-	-	-

***Chrysochromulina pringsheimii* Parke et Manton** **Figs. 4.56 - 4.60**

Micrographs: Parke and Manton, 1962; Plates 2 - 6.
Beech, 1983; Plate 2.7.
Hallegraeff, 1983; Fig. 14.

Present Findings.
Scales were found in samples from the Derwent River, Crayfish Point, Pipeclay Lagoon, Oyster Cove Point, Eaglehawk Neck and Honey Moon Bay.

Scales and whole cells were found in enrichment cultures (GSe/10 and ML (+GeO_2) media) derived from Dru Point samples. Individual cells were isolated and a unialgal culture was successfully maintained in the CSIRO Collection of Living Microalgae for three years.

Description.
Cells were approximately 10 x 5 µm. Unfortunately, the flagella and haptonema became detached from cells during sample preparation and no whole cells were observed under TEM. Part of the haptonema is seen in Fig. 4.57. Four different scale types were identified.

Small oval plate scales, 0.8 x 0.4 µm (n=2), were few in number and only seen from enrichment cultures. They had a pattern of c. 60 radiating ridges arranged in quadrants and extending to the scale edge on one surface, and faint concentric fibrils on the opposite surface (Fig. 4.57). These scales matched those described for the type species (Parke and Manton, 1962).

Larger oval plate scales, 0.9 - 1.4 x 0.7 - 1.0 µm ($\bar{x}$= 1.1 x 0.9 µm; n=19), were more numerous and observed from both wild samples and enrichment cultures. They were slightly smaller than previous reports (Table 4.24), but had similar surface patterning. Approximately 80 radiating ridges were arranged in quadrants, in comparison to c. 120 ridges in the scales described by Parke and Manton (1962; Fig. 25) and by Beech (1983; Plate 2.7B). These ridges extended to the scale edge on one surface and to a peripheral band of faint concentric fibrils on the opposite surface (Fig. 4.56).

Small spine scales were also common and found in wild samples and enrichment cultures. These scales consisted of four raised struts, extending from the rim of a concave base plate and fusing to form a short central spine (Fig. 4.60), 0.5 - 0.9 µm

long ($\bar{x}$=0.7 µm; n=25). This length was shorter than that given in the original description (Parke and Manton, 1962), but similar to other Australian material (Table 4.24). The struts and spine were often seen collapsed against the base plate, which was patterned on both surfaces with 72 - 76 slightly curved radiating ridges arranged in quadrants and extending to a conspicuous rim (Figs. 4.56, 4.57). The base plate dimensions ranged from 1.0 - 2.0 x 0.9 - 1.5 µm ($\bar{x}$=1.3 x 1.1 µm; n=18), marginally smaller than previous measurements (Table 4.24).

Long spine scales were occasionally found, more often from enrichment cultures than from wild samples. These had a similar construction to the smaller spine scales, but with a much longer central spine and thicker supporting struts (Fig. 4.58). The spine had a variable length, ranging from 8 - 26 µm ($\bar{x}$=8.6 µm; n=7), and was generally straight, although a curved spine was also found (Fig. 4.58). The base plate, 1.3 - 3.1 µm diameter ($\bar{x}$=1.9 µm; n=5), had a thick rim and was usually folded allowing the characteristic pattern of radiating ridges to be distinguished (Fig 4.59). Fine longitudinal striations were seen along the spine. Five to six of these long spine scales were observed per cell, which agreed with the type description (Parke and Manton, 1962).

There was little variation in *C. pringsheimii* scale size and structure from enrichment cultures and from wild samples. However, for both types of spine scales, spine length was generally longer in wild samples. For the small spine scales, spine length ranged from 0.5 - 0.9 µm (n=8) in wild material and from 0.5 - 0.6 µm (n=10) in enrichments. For the larger spine scales, spine length was 26 µm in wild material and ranged from 7.5 - 10 µm (n=6) in enrichments.

Distribution.

C. pringsheimii has been reported from temperate coastal waters in both hemispheres, including those of: UK (Parke and Manton, 1962), Norway (Leadbeater, 1972b), Denmark (Manton and Leadbeater, 1974), Yugoslavia (Leadbeater, 1974), New Zealand (Rhodes and Burke, 1996) and Australia (Beech, 1983; Hallegraeff, 1983).

It has also been recorded from the North Atlantic Ocean (Estep et al, 1984), and spine scales have been found in tropical Australian waters (Hallegraeff, 1983).

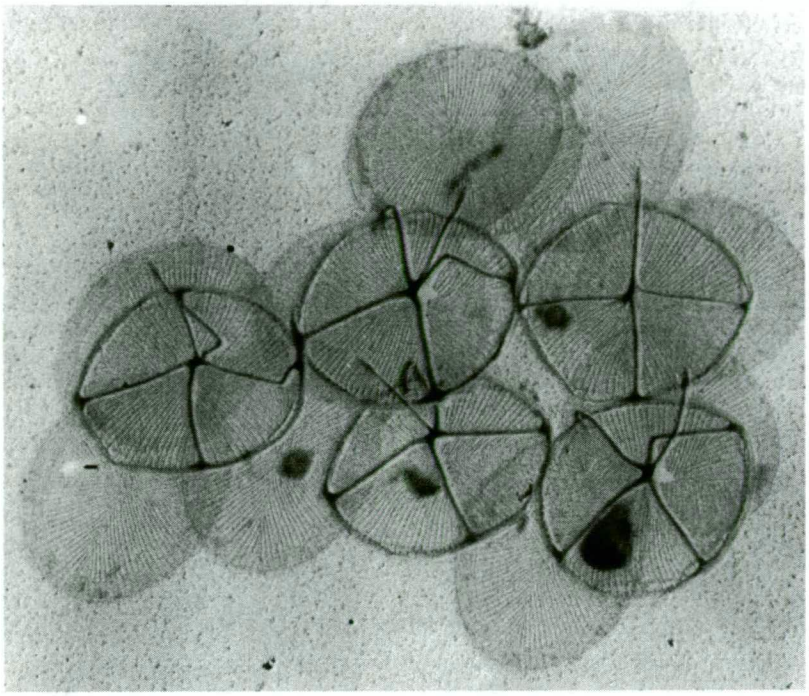


Fig.4.56: *C. pringsheimii* plate and spine scales (c. 1 μm); from a Dru Point enrichment culture

(Micrograph no: 5242)

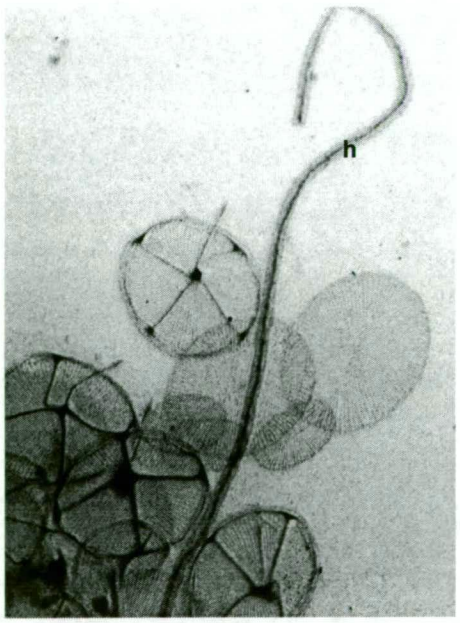


Fig. 4.57: *C. pringsheimii* plate and spine scales (c. 1 μm), and small plate scales (0.8 x 0.4 μm); part of the haptonema (h) also shown; from a Dru Point enrichment culture

(Micrograph no: 5243)

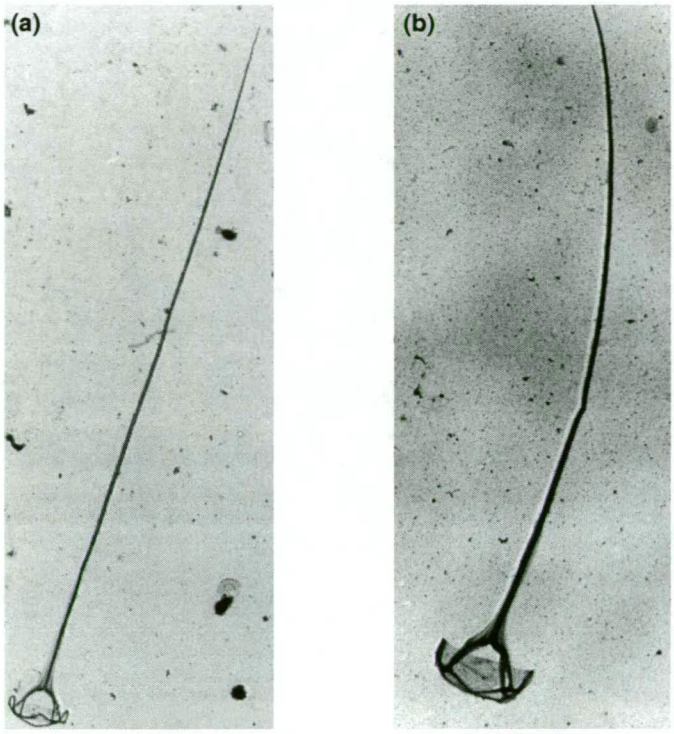


Fig. 4.58: *C. pringsheimii* long spine scales: (a) straight spine, (20 μm) from Dru Point enrichment; (b) curved spine (10 μm), from Eaglehawk Neck

(Micrograph no: 5251, 5410)

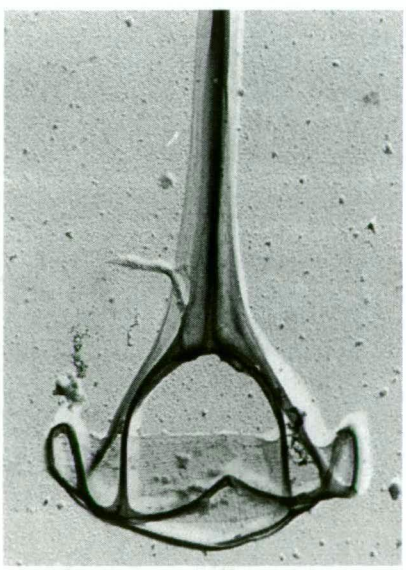


Fig. 4.59: *C. pringsheimii,* detail of long spine scale base (c. 2 μm); from Eaglehawk Neck

(Micrograph no: 5411)

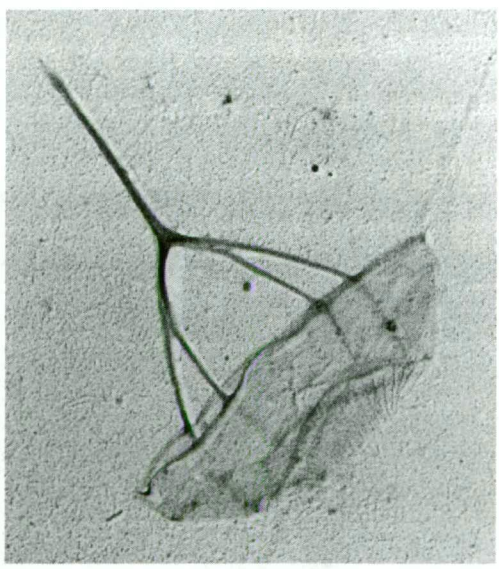


Fig. 4.60: *C. pringsheimii* small spine scale (c. 1 μm base) showing struts supporting spine; from Pipeclay Lagoon

(Micrograph no: 5448)

Table 4.24: Scales of *Chrysochromulina pringsheimii* from different locations.

SOURCE	SMALL PLATES		LARGE PLATES		SMALL SPINES		LARGE SPINES		
	Dimensions (µm)	*No. of Ridges*	*Dimensions (µm)*	*No. of Ridges*	*Dimensions (µm)*	*Height (µm)*	*No. of Ridges*	*Dimensions (µm)*	*Height (µm)*
UK (Parke & Manton, 1962)	0.8 x 0.5	c. 60	1.7 x 1.3	c. 120	1.9 x 1.4	1.0 - 1.5	c. 100	c. 2	12 - 20
Victoria, Australia (Beech, 1983)	0.7 x 0.5 (n=2)	c. 60	1.8 x 1.5 (n=3)	c. 120	1.8 x 1.2 (n=2)	0.8 (n=20)	c. 100	1.8 (n=1)	25 (n=1)
NSW, Australia (Hallegraeff, 1983)	-	-	-	-	1.8 x 1.3	0.6	c. 80	-	-
Gulf of Carpentaria, Australia (Hallegraeff, 1983)	-	-	-	-	-	-	-	1.5	14
Tasmania, Australia	0.8 x 0.4 (n=2)	c. 60	0.9 - 1.4 x 0.7 - 1.0 ($\bar{x}$ =1.1 x 0.9; n=19)	c. 80	1.0 - 2.0 x 0.9 - 1.5 ($\bar{x}$ =1.3 x 1.1; n=18)	0.5 - 0.9 ($\bar{x}$ =0.7; n=25)	72 - 76	1.3 - 3.1 ($\bar{x}$ =1.9; n=5)	8 - 26 ($\bar{x}$ =8.6;n=7)

***Chrysochromulina pyramidosa* Thomsen** **Figs. 4.61 - 4.62**

Micrographs: Thomsen, 1977; Figs. 1 - 2.
Hallegraeff, 1983; Fig. 15.
Beech, 1983; Plate 2.8 A - B.
Thomsen, 1986; Figs. 21 - 22.

Present Findings.
Whole cells and scales were found in Southport and Honeymoon Bay samples.

Whole cells and scales were also found in enrichment cultures derived from Derwent River (GSe and ML (+GeO_2) media) and Dru Point (GSe/10 (+GeO_2) medium) samples.

Description.
Whole cells (4 x 3 µm) had two equal flagella, 9 - 13 µm long ($\bar{x}$=11; n=4) and a slightly shorter haptonema, 5 - 8 µm long ($\bar{x}$=7; n=2) (Fig. 4.61). These measurements were similar for both cultured and collected material, and agreed with previous descriptions of this species (Table 4.25).

Two scale types were found: circular plate scales and distinctive pyramid scales (Fig. 4.62). Plate scales were 0.6 - 0.7 µm ($\bar{x}$=0.6 µm; n=13), and were patterned with 24 - 26 evenly-spaced radiating ridges overlying 7 - 8 equidistant concentric rings. Pyramid scales had four struts, 0.6 - 0.7 µm long ($\bar{x}$=0.6 µm; n=11), extending from the scale edge and converging at a point above the scale, thus forming a pyramid-like structure. The scale base was circular and slightly larger than the plate scales, ranging from 0.7 - 0.9 µm diameter ($\bar{x}$=0.8; n=11), but with similar surface patterning (26 - 28 radiating ridges and 8 concentric rings). In both scale types, the outermost concentric ring was thicker than the inner rings.

Scale sizes were similar for cultured cells and wild material, and both scale size and structure agreed with the type description (Thomsen, 1977). There were few differences in *C. pyramidosa* scales described from different locations (Table 4.26).

Distribution.
C. pyramidosa has been previously reported from Denmark, South Africa and south-east Australia (Thomsen, 1977; Beech, 1983; Hallegraeff, 1983).

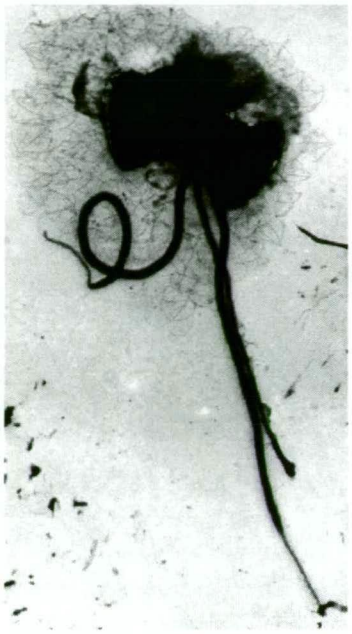


Fig. 4.61: *C. pyramidosa* cell, (4 x 3 μm); from a Derwent River enrichment culture

(Micrograph no: 5281)

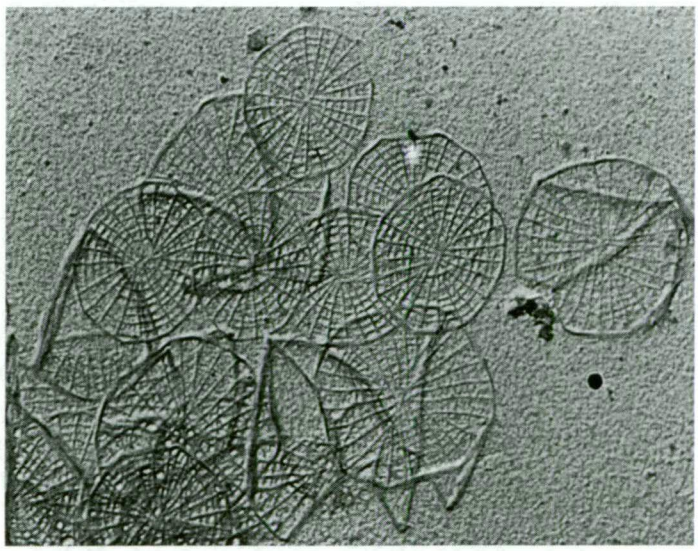


Fig. 4.62: *C. pyramidosa* pyramid and plate scales (c. 0.6 μm); from Coles Bay

(Micrograph no: 5510)

Table 4.25: ***Chrysochromulina pyramidosa*** **cells from different locations.**

SOURCE	CELL DIAMETER (μm)	FLAGELLAR LENGTH (μm)	HAPTONEMA (μm)
Denmark (Thomsen, 1977)	4 x 3	10 - 13	5 - 6
NSW, Australia (Hallegraeff, 1983)	3.5	-	-
Victoria, Australia (Beech, 1983)	c. 4 (n=1)	c. 15 (n=1)	-
Tasmania, Australia	4 x 3 (n=1)	9 - 13 ($\bar{x}$=11; n=4)	5 - 8 ($\bar{x}$=7; n=2)

Table 4.26: Scales of *Chrysochromulina pyramidosa* scales from different locations.

SOURCE	PLATE SCALES			PYRAMID SCALES			
	Diameter (µm)	*No. of Ridges*	*No. of Rings*	*Diameter (µm)*	*No. of Ridges*	*No. of Rings*	*Strut Length*
Denmark (Thomsen, 1977)	c. 0.7	24	7 - 8	0.8 - 0.9	27 - 28	8 - 9	0.6 - 0.8 ($\bar{x}$ =0.72, n=4)
NSW, Australia (Hallegraeff, 1983)	0.6 - 0.7	21	6 - 7	-	-	-	0.6
Victoria, Australia (Beech, 1983)	0.63 - 0.67 ($\bar{x}$ =0.6, n=4)	23	7 - 8	-	-	-	0.6 - 0.7 ($\bar{x}$ =0.67, n=2)
Tasmania, Australia	0.57 - 0.67 ($\bar{x}$ =0.6, n=13)	24 - 26	7 - 8	0.71 - 0.88 ($\bar{x}$ =0.8, n=11)	26 - 28	8	0.6 - 0.7 ($\bar{x}$ =0.64, n=11)

***Chrysochromulina* aff. *scutellum* Eikrem et Moestrup** **Figs. 4.63 - 4.66**

Micrographs: Eikrem and Moestrup, 1998; Figs. 11 - 14.
Hadju et al, 1996; Fig. 4D.

Present Findings.
Whole cells and scales were found in samples from Pipeclay Lagoon and Southport, and were also identifed from a GSe enrichment culture derived from a Derwent River sample. Individual cells were isolated and a unialgal culture (CS-496) is currently maintained in the CSIRO Collection of Living Microalgae (in GSe medium at 15°C, under standard growth conditions).

This is a new record for Australian waters, and the first report from the southern hemisphere. It is also the first report of this species since its original description (Eikrem and Moestrup, 1998).

Description.
Cells were approximately 5 µm, with two flagella, 16 - 20 µm, and a coiled haptonema (Fig. 4.63).

C. scutellum has three scale types (Eikrem and Moestrup, 1998), two of which were clearly identified from the Tasmanian material. The large oval scales were characteristic of this species, while the two types of small spine scales could be easily confused with those of *C. ephippium*, *C. alifera* or *C. kappa.*

The larger scale type was oval and slightly compressed, 0.6 - 0.7 x 0.4 - 0.5 µm ($\bar{x}$=0.65 x 0.42 µm; n=4), with a thickened margin (Fig. 4.64). Evenly-spaced radiating ridges (c. 56) extended from a patternless central area to the scale margin. Eikrem and Moestrup (1998) described a concentric fibrillar pattern on the distal surface (Figs. 11 and 14), but scales with this pattern were not observed in this study.

The smaller scale type had a short central spine, 0.15 µm long (n=2), attached to the scale base by four decurrent struts which extended to an upright rim. The base, 0.25 - 0.35 µm diameter ($\bar{x}$=0.36 µm; n=5), had a pattern of c. 24 radiating ridges, arranged in quadrants, and an outer upright rim (c. 0.05 µm; n=2). This pattern of radiating ridges was seen on both scale surfaces (Figs. 4.65, 4.66). However, in the material described by Eikrem and Moestrup (1998), a concentric fibrillar pattern on the distal surface was illustrated (Fig. 12).

The third scale type, another spine scale (diameter c. 0.3 μm; spine height c. 0.15 μm; n=1), but without an upright rim, was found at the cell surface (Fig. 4.64). A faint surface patterning of radiating ridges overlying concentric fibrils could be seen, but it was difficult to distinguish further details.

Distribution.

C. scutellum was originally described from cultured material from the Skagerrak, between Norway and Denmark (Eikrem and Moestrup, 1998), and from wild samples collected from the Baltic Sea, south of Stockholm (Hadju et al, 1986).

Toxicity.

C. scutellum was found to be non-toxic to *Artemia* nauplii (Edvardsen, 1993; Eikrem and Moestrup, 1998). However, in the present study, *Artemia* nauplii fed stationary phase cultures of *C.* aff. *scutellum* markedly slowed their swimming activity (see Chapter 7).

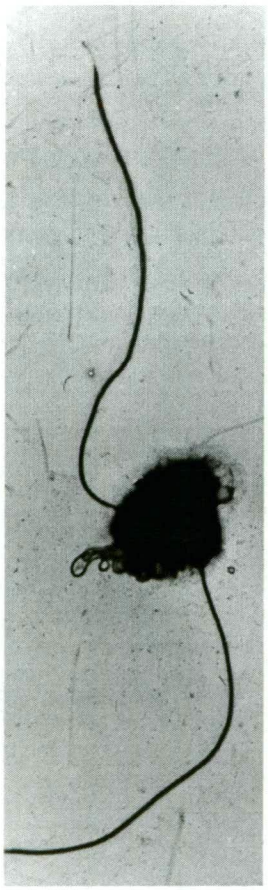


Fig. 4.63: *C.* aff. *scutellum* cell (c. 5 μm) showing two long flagella and coiled haptonema; from Pipeclay Lagoon

(Micrograph no: 5437)

Fig. 4.64: *C.* aff. *scutellum* large, oval scales (c. 0.6 x 0.4 μm) and small rimless spine scales (c. 0.3 μm); from Derwent River enrichment culture

(Micrograph no: 4842)

Fig. 4.65: *C.* aff. *scutellum* spine scales (0.35 μm) - proximal view; from Pipeclay Lagoon

(Micrograph no: 5441)

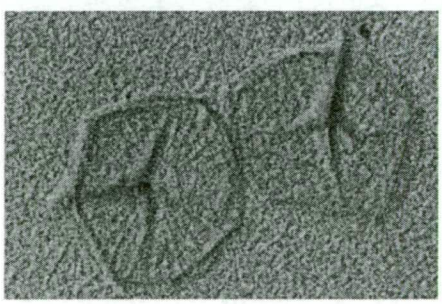


Fig. 4.66: *C.* aff. *scutellum* spine scales (0.35 μm) - distal and proximal views; from Pipeclay Lagoon

(Micrograph no: 5441)

***Chrysochromulina simplex* Estep, Davis, Hargraves et Sieburth *emend.* Birkhead et Pienaar** **Fig. 4.67**

Micrographs: Birkhead and Pienaar, 1995b; Figs. 11 - 13
Moestrup, 1979; Fig. 26.
Estep et al, 1984; Fig. 15.
Beech, 1983; Plate 2.11 A - C.
Hadju et al, 1996: Fig. 4C.
Rhodes and Burke, 1996; Fig. 5.

Present Findings.
Scales were observed in a Derwent River sample, and a whole cell was found in the Deep Bay sample.

Whole cells and scales were found in five enrichment cultures, all established from Derwent River samples in GSe or GSe/2 medium.

This species was successfully isolated by micromanipulation and is currently maintained in the CSIRO Collection of Living Microalgae (as CS-483) in GSe medium at 15°C, under standard growth conditions.

Description.
Cells were approximately 3 µm with two flagella, c. 10 - 15 µm, and a long coiling haptonema.

C. simplex has only one scale type - a circular to slightly oval plate with different patterning on each surface (Fig. 4.67). Scales ranged from 0.29 - 0.40 µm in diameter ($\bar{x}$=0.33 µm, n=58), with little variation in size of wild and cultured cells (Table 4.27). On the distal scale surface, a pattern of concentric rings was observed. The proximal surface had 22 - 26 evenly-spaced radiating ridges, superimposed on numerous fine concentric rings, and a central cross. These ridges extended from a patternless central area to the scale edge. Scale size and structure of the Tasmanian material agreed with the emended description of the species given by Birkhead and Pienaar (1995b).

Scale size and structure were fairly uniform in a range of material, both from wild samples and cultures, and from different locations (Table 4.27). As the original type description included three different forms of scale patterning (Estep et al, 1984),

there has been some confusion in correctly identifying this species. Descriptions of *Chrysochromulina* sp. by Moestrup (1979; Fig. 26) and Beech (1983; Plate 2.11 A - C) agreed with the emended description of *C. simplex*, whereas scales illustrated by Hallegraeff (1983; Fig. 18a) and Estep et al (1984; Figs. 16 - 18) were much larger with different surface patterning.

Distribution.

C. simplex was first isolated from the English Channel and maintained in the Plymouth Culture Collection (as PCC-384). It has since been reported mostly from the southern hemisphere, namely New Zealand (Moestrup, 1979; Rhodes and Burke, 1996), south-east Australia (Beech, 1983), South Africa and the southern Indian Ocean (Birkhead and Pienaar, 1995b), but has also been recorded from the North Atlantic Ocean (Estep et al, 1984). In addition, Smith and Hobson (1994) reported *C. simplex* from a temperate Canadian fjord; however, no micrographs were published to confirm species identification.

Toxicity.

C. simplex has been reported as non-toxic to *Artemia* nauplii (Simonsen and Moestrup, 1997), which agreed with findings in this study (see Chapter 7).

Fig. 4.67: *C. simplex* scales (c. 0.4 μm) - proximal and distal views; from a Derwent River enrichment culture

(Micrograph no: 4660)

Table 4.27: Scales of *Chrysochromulina simplex* from different locations.

SOURCE	MATERIAL EXAMINED	SCALE DIAMETER	NO. OF RADIATING RIDGES
North Atlantic Ocean (Estep et al, 1984)	Wild	0.30 - 0.52 ($\bar{x}$ =0.42, n=5)	27 (n=1)
New Zealand (Moestrup, 1979)	Wild	0.40 - 0.46 ($\bar{x}$ =0.42, n=7)	30-34 (n=4)
New Zealand (Rhodes & Burke, 1996)	Cultured	0.33 - 0.43 (circular) 0.27 - 0.42 x 0.23 - 0.39 (ellipsoid)	23 - 25
S. Indian Ocean (Birkhead & Pienaar, 1995b)	Cultured	0.34 - 0.51	21 - 26
Victoria, Australia (Beech, 1983)	Cultured	0.30 - 0.40	22 - 24 (n=2)
Tasmania, Australia	Wild	0.29 - 0.33 ($\bar{x}$ =0.31; n=5)	c. 20 (n=5)
	Cultured	0.30 - 0.40 ($\bar{x}$ =0.35; n=53)	22 - 26 (n=20)

***Chrysochromulina* cf. *simplex* (Estep, Davis, Hargraves et Sieburth) emend. Birkhead et Pienaar** **Figs. 4.68 - 4.70**

Micrographs: Hallegraeff, 1983; Fig 18a.

Present Findings.

Scales similar to *C. simplex* were found in Derwent River and Dru Point samples.

Description.

Scales were circular, 0.7 - 0.9 μm in diameter (n=4), with a similar patterning to *C. simplex*, namely proximal radiating ridges overlying faint concentric rings, a central cross, and distal concentric bands (Figs. 4.68 - 4.70). However, these scales were almost double the size of *C. simplex* scales, and had more than twice the number of radiating ridges on the proximal surface (approximately 50 - 60). Unlike the straight ridges of *C. simplex,* the ridges were slightly curved.

Scales appeared to similar to those described by Hallegraeff (1983; Fig. 18) from the East Australian Current; these were 1.0 - 1.2 μm in diameter with 44 - 80 proximal radiating ridges, also slightly curved.

Fig. 4.68: *C.* aff. *simplex* plate scales (0.9 μm); from the Derwent River

(Micrograph no: 4907)

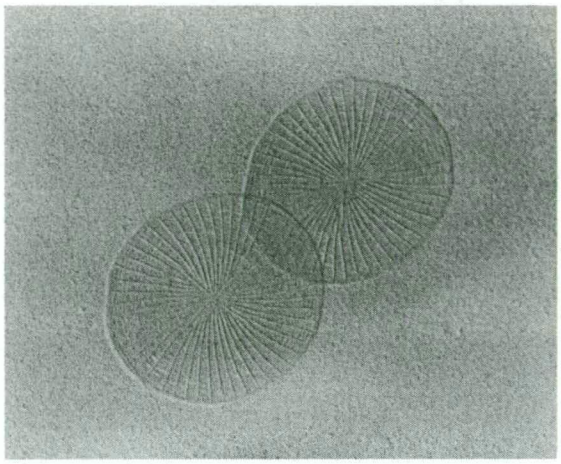


Fig. 4.69: *C.* aff. *simplex* plate scales (0.7 μm) - proximal view; from Dru Point

(Micrograph no: 4962)

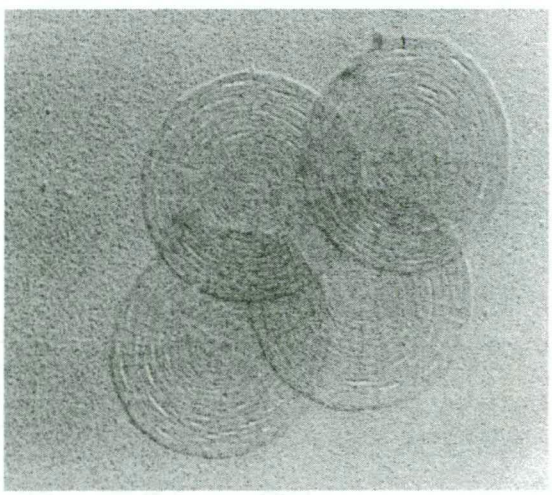


Fig. 4.70: *C.* aff. *simplex* plate scales (0.7 μm) - distal view; from Dru Point

(Micrograph no: 4963)

***Chrysochromulina spinifera* (Fournier) Pienaar et Norris** **Figs. 4.71 - 4.72**

Micrographs: Pienaar and Norris; 1979. Figs. 2 - 9.
Moestrup, 1979; Figs. 19, 23, 25.
Beech, 1983; Plate 2.8 C - E

Present Findings.
Scales were found in samples collected from the Derwent River, Oyster Cove Point, Southport, Pipeclay Lagoon and Honey Moon Bay. Whole cells were also seen in a Pipeclay Lagoon sample.

Chrysochromulina spinifera grew well in GSe enrichment cultures established from the Derwent River, Oyster Cove Point, Fleurty Point and Deep Bay, as well as in a GSe/10 enrichment culture from Little Swanport.

Description.
Cell size was approximately 8 x 6 µm. There were two unequal flagella (26 - 38 µm; n=1) and a short haptonema (11 µm; n=1), which were significantly longer than previously reported (Table 4.28). In this study, measurements were made of a shadow cast whole cell in which the flagella and haptonema were detached from the cell, whereas the comparative measurements were made of live cells under the light microscope (Fournier, 1971; Pienaar and Norris, 1979). This may explain the differences observed in flagellar and haptonemal lengths.

Two different scale types were seen. Fig. 4.71 shows an empty scale case with long spine scales and numerous small oval plate scales.

Spine scales were 16 - 26 µm long ($\bar{x}$=20.8 µm; n=6) and 0.2 µm wide (n=2), with a base width ranging from 1.0 - 1.2 µm ($\bar{x}$=1.1; n=7) (Fig. 4.72). Each spine scale had numerous longitudinal fibrils extending the length of the spine, a characteristic four-pointed tip and a truncated base (Figs. 4.73, 4.75). The fine cross fibrils mentioned by Pienaar and Norris (1979; Fig. 4) were not seen in the Tasmanian material.

The number of spine scales per cell was highly variable. A scale case from the Derwent River had nine spine scales in comparison to c. 40 spine scales found on a cell from a Deep Bay enrichment culture.

Oval plate scales were 0.8 - 1.0 x 0.6 - 0.9 μm ($\bar{x}$=0.9 x 0.7 μm; n=23) and had a distinctly different pattern on each surface. The proximal surface had a series of slightly curved radiating ridges, arranged in quadrants and extending to the scale edge, superimposed on loosely woven concentric fibrils. The distal surface had irregularly arranged fibrils, and a broad peripheral band with a concentric pattern (Figs. 4.73, 4.74).

Scale size and structure generally corresponded to the description given by Pienaar and Norris (1979), and agreed with other reports of this species (Table 4.28). The greatest variation existed in the number of spine scales reported per cell and the spine scale length. Spine length was generally longer in wild material in comparison to cultured cells, which agreed with the observations made for another spine-bearing species, *C. pringsheimii.* There was also an overall trend towards more spine scales on cultured cells in comparison to wild material.

Distribution.

C. spinifera has been reported from temperate waters in both hemispheres, including those of Canada, USA, England, South Africa (Pienaar and Norris, 1979, and references therein), Denmark (Knipschildt, 1992), New Zealand (Moestrup, 1979) and south-east Australia (Beech, 1983).

Toxicity.

C. spinifera was one of five species in a dense *Chrysochromulina* bloom in Danish coastal waters, which resulted in the death of several tons of farmed rainbow trout (Knipschildt, 1992; Hansen et al, 1995). As mentioned previously, the long spines of *C. spinifera* and the two other spine-bearing species involved in the bloom, *C. ericina* and *C. hirta*, may have caused sufficient gill damage to result in fish mortality.

In the present study, *C. spinifera* was not obtained in unialgal culture, and hence could not be tested for toxicity.

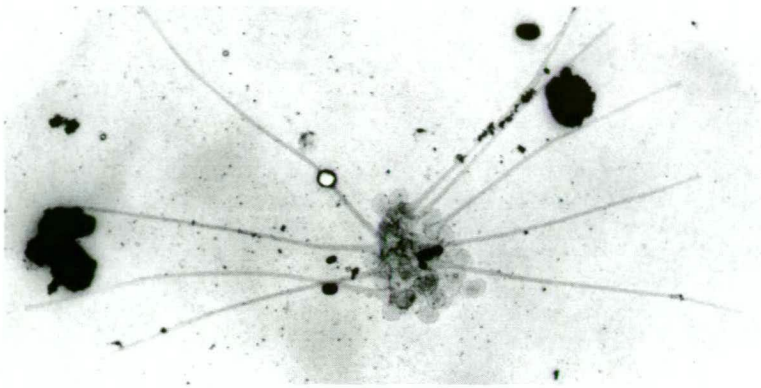


Fig. 4.71: *C. spinifera* scale case; from the Derwent River

(Micrograph no: 5229)

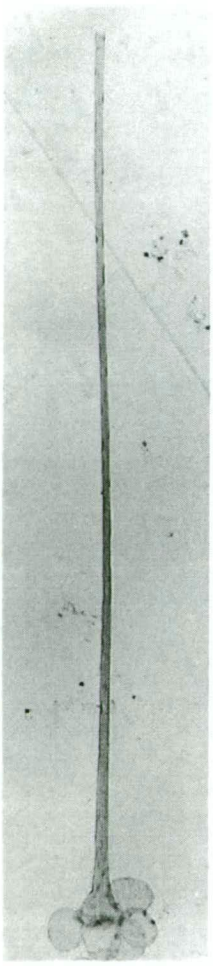


Fig. 4.72: *C. spinifera* spine scale (20 μm); from a Derwent enrichment culture

(Micrograph no: 4922)

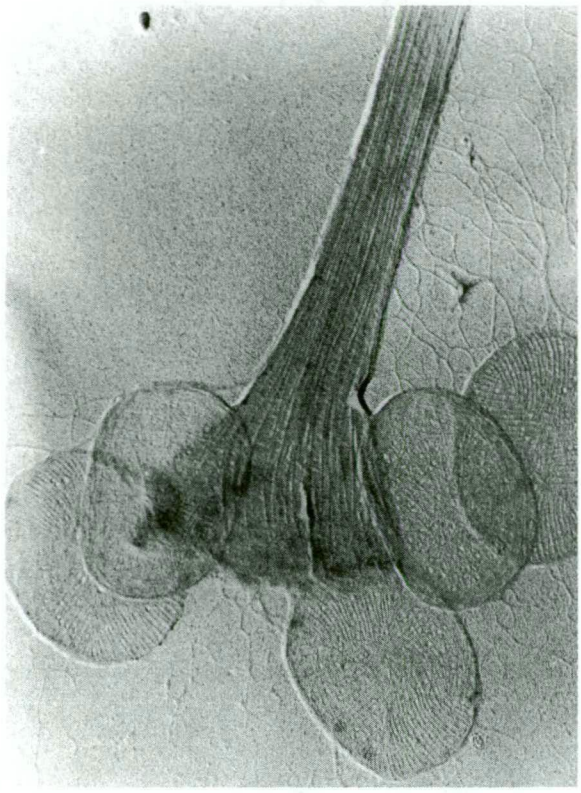


Fig. 4.73: *C. spinifera* spine scale, showing striated spine, and plate scales (0.9 x 0.7 μm); from Dru Point

(Micrograph no: 4820)

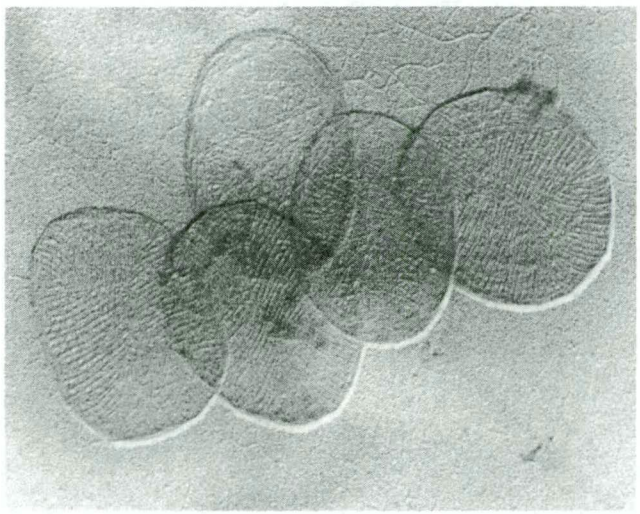


Fig. 4.74: *C. spinifera* plate scales - proximal and distal views; from a Derwent River enrichment culture

(Micrograph no: 4925)

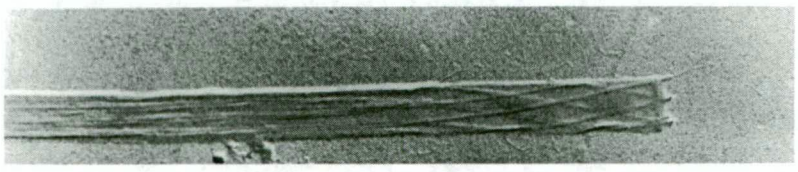


Fig. 4.75: Detail of *C. spinifera* spine tip; from the same culture

(Micrograph no: 4918)

Table 4.28: *Chrysochromulina spinifera* cells from different locations.

SOURCE	CELL SIZE	FLAGELLA		HAPTONEMA	SPINE SCALES			PLATE SCALES	
	(µm)	*Long (µm)*	*Short (µm)*	*(µm)*	*Length (µm)*	*Base Width (µm)*	*No. per Cell*	*Length (µm)*	*Width (µm)*
Canada* (Fournier, 1971)	6 - 10 x 3 - 9	11 - 30	7 - 12	4 - 8	ND	ND	ND	ND	ND
USA** *(type)* (Pienaar & Norris, 1979)	8 - 10 x 7 - 9	11.5	6.7	4 - 5	10 - 18	1.0	40 - 50	0.6 - 1.06	0.8
New Zealand* (Moestrup, 1979)	ND	ND	ND	ND	21 - 36	1.0	23 - 31	1.1	0.9
Victoria, Australia* (Beech, 1983)	ND	ND	ND	ND	20 (n=1)	1.3 (n=1)	ND	1.1 (n=1)	0.9 (n=1)
Tasmania, Australia									
- Wild material	8.0 x 6.0 (n=1)	38 (n=1)	26 (n=1)	11 (n=1)	16 - 26 ($\bar{x}$ =20.8; n=3)	1.2 ($\bar{x}$ =1.2; n=3)	9	ND	ND
- Cultured cells	ND	ND	ND	ND	20 - 21 ($\bar{x}$ =20.8; n=3)	1.0 - 1.1 ($\bar{x}$ =1.1; n=4)	c. 40	0.8-1.0 ($\bar{x}$ =0.9, n=23)	0.6-0.9 ($\bar{x}$ =0.7, n=23)

ND = Not determined *Wild material ** Cultured cells

***Chrysochromulina* aff. *vexillifera* Manton et Oates** **Figs. 4.76 - 4.77**

Micrographs: Manton and Oates, 1983; Figs. 2 - 16.
Beech, 1983; Plate 2.9.
Estep et al, 1994; Figs. 22, 23.
Hoepffner and Haas, 1990; Fig. 7.

Present Findings.
Scales were found in a Pipeclay Lagoon sample.

Description.
Three scale types were found which resembled those of *Chrysochromulina vexillifera.*

Spine scales were long and slender, 7.8 - 8.0 µm in length (n=2), each tapered to a pointed end. Spines were attached to an apparently convex base plate, 0.8 - 0.9 µm in diameter (n=2), and supported by four struts (Fig. 4.76). The base plate was patterned with numerous radiating ridges and had a narrow perforated margin. These scales were similar to those described by Manton and Oates (1983) for the type species. Spine scales from different locations varied in size, with spine length ranging from 4 - 22 µm and base plate diameter from 0.5 - 1.5 µm (Table 4.29).

Oval plate scales, 0.9 - 1.0 x 0.6 - 0.7 µm in diameter ($\bar{x}$=0.92 x 0.68 µm; n=5), had a very short central spine and were divided into quadrants by four distinct ridges, which formed a central oblique cross (Fig. 4.77). These ridges were consistently orientated so that the wide angle of the cross was directed towards the scale ends, and the narrow angle of the cross faced the long sides. Each scale was patterned with faint radiating ridges which extended to a narrow scale margin. A few slightly smaller plate scales, 0.8 - 0.6 µm (n=1), lacking the central cross but otherwise with similar patterning, were also seen.

C. vexillifera plate scales have the distinct central cross but lack the short spine and the scale margin seen in the Tasmanian material (Manton and Oates, 1983; Figs. 7, 9, 13, 14). The cross orientation is also different, with the wide angle facing the scale sides and the narrow angle directed towards the scale ends. Scales are twice the size of those found in this study and have a distinct pattern of numerous radiating ridges (c. 90 - 140). However, other plate scales illustrated by Manton and Oates (1983; Fig. 17) as having similarities to *C. vexillifera*, closely matched the Tasmanian scales.

Like the spine scales, *C. vexillifera* plate scales from different locations also varied in size, with the larger plate scales ranging from 0.8 - 2.0 μm, and the smaller plate scales lacking the distinctive cross, from 0.5 x 0.4 - 0.8 x 0.5 μm (Table 4.29).

The long tapering spine scales and the oblique cross on the plate scales are characteristic of this species. Scales with these features from Sydney coastal waters, recorded by Hallegraeff (1983; Fig. 13) as *C.* aff. *latilepis*, were actually *C. vexillifera.*

Distribution.

C. vexillifera has been reported from the Galapagos Islands (Manton and Oates, 1983), the North Atlantic Ocean (Estep et al, 1984), the North Pacific Ocean (Hoepffner and Haas, 1990) and from south-east Australia (Beech, 1983).

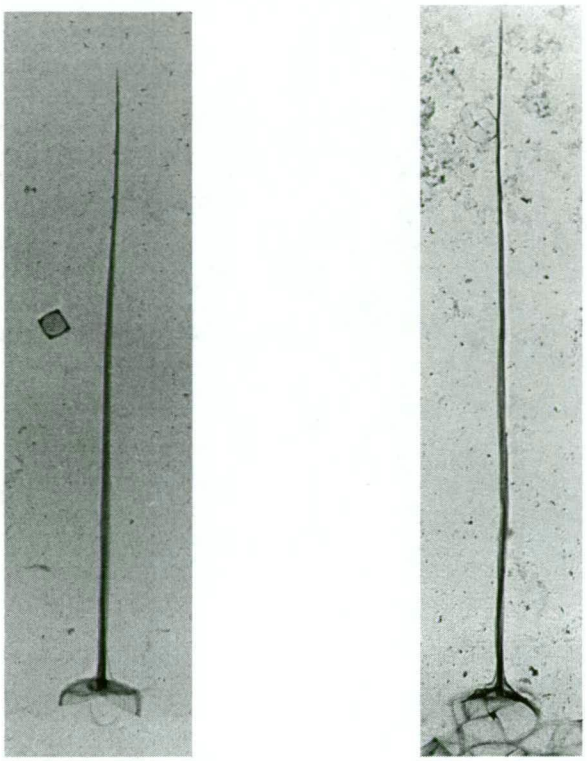


Fig. 4.76: *C.* aff. *vexillifera* spine scales (c. 8 μm long); from Pipeclay Lagoon

(Micrograph no: 5455, 5454)

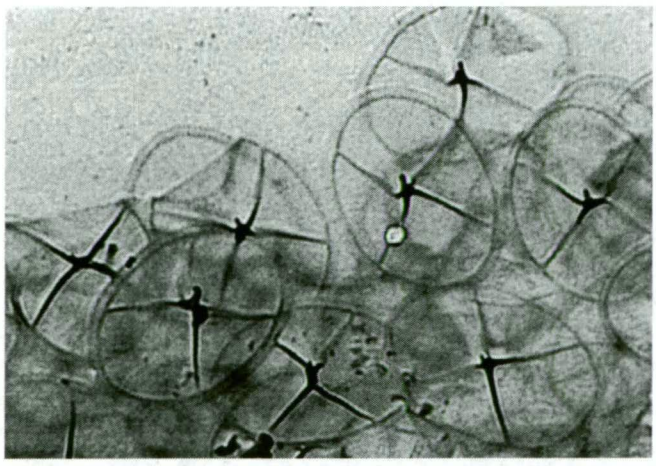


Fig. 4.77: *C.* aff. *vexillifera* plate scales (c. 0.9 x 0.7 μm), showing central cross and short spine; from Pipeclay Lagoon

(Micrograph no: 5454)

Table 4.29: Scales of *Chrysochromulina vexillifera* from different locations.

SOURCE	SPINE SCALES		PLATE SCALES WITH CROSS	PLATE SCALES WITHOUT CROSS
	Length (µm)	*Base Diam. (µm)*	*Dimensions (µm)*	*Dimensions (µm)*
C. vexillifera				
Galapagos Islands (Manton & Oates, 1983)	≤ 15	0.9 (n=1)	≤ 2	0.8 x 0.5
Victoria, Australia (Beech, 1983)	18 - 22	c. 1.5 (n=1)	1.8 - 2.0	-
New South Wales, Australia (Hallegraeff, 1983)	5	0.5 (n=1)	1.0 - 1.3	0.7 x 0.5
North Atlantic Ocean (Estep et al, 1984)	7 (n=1)	c. 1.0 (n=1)	1.5 - 1.7	0.8 - 0.7 (n=2)
North Pacific Ocean (Hoepffner & Haas, 1990)	4 (n=1)	0.5 (n=1)	0.9 x 0.8 (n=2)	0.5 x 0.4 (n=1)
C.* aff. *vexillifera				
Tasmania, Australia	7.8 - 8.0 (n=2)	0.8 - 0.9 (n=2)	0.9 - 1.0 x 0.6 - 0.7 ($\bar{x}$ =0.92 x 0.68; n=2)	0.8 x 0.6 (n=1)

***Chrysochromulina* sp. "eyelash"** **Figs. 4.78 - 4.82**

Micrographs: Moestrup, 1979; Fig. 24.
Beech, 1983; Plate 2.13 E - F.
Pienaar and Bandu, 1984; Figs. 2 - 11.
Birkhead and Pienaar, 1995a; Fig. 2.

This species of *Chrysochromulina* is distinguished by its beautifully elaborate "eyelash" scales, but it has not yet been formally described.

Present Findings.
Scales were found in water samples from the Derwent River, Crayfish Point, Dru Point and Port Huon.

Whole cells and scales were found in enrichment cultures (GSe and GSe/2 media) established from two Derwent River samples. Scales were found in enrichment cultures derived from a Dru Point sample (GSe/10 (+GeO_2) medium) and from a Maria Island sample (ML (+GeO_2) medium).

This species was successfully isolated from the Derwent River enrichment cultures. Two strains (CS-410/8, 10) are currently maintained in the CSIRO Living Collection of Microalgae (in GSe medium at 15°C, under standard growth conditions).

Description.
Whole cells were approximately 10 µm. They had two flagella, ranging from 20 - 25 µm in length, and a short haptonema, about 10 µm. These measurements agreed with those given by Pienaar and Bandu (1984).

This species had three different types of scales: small and large plate scales, and the distinctive "eyelash" scales (Fig. 4.78).

The large plate scales were 0.8 - 1.0 x 0.6 - 0.8 µm in size ($\bar{x}$=0.9 x 0.7 µm; n=26), and were more numerous than the smaller plate scales. These smaller scales were approximately half the size of the large plate scales, at 0.5 x 0.4 µm (n=7). Both scale types were round to oval, with similar surface patterning. The proximal surface had numerous radiating ridges, arranged in quadrants and overlying faint concentric rings. The number of ridges per quadrant was c. 24 for the large plate scales and 14 for the small plate scales. The ridges extended to the scale edge which was

patterned with small pores (Figs. 4.81, 4.82). On the distal surface, there was faint pattern of radiating ridges and concentric rings, with an amorphous central area and a broad peripheral band (Figs. 4.80, 4.82).

The "eyelash" scales were first described by Moestrup (1979; Fig. 24) from a partially-obscured scale which did look like an eyelash. However, when the entire scale was seen in later samples, it looked more like a small sunshine! Scales were circular to elliptical, 0.6 - 0.8 µm in size ($\bar{x}$=0.7 µm; n=40). A complex central spine, 0.4 - 0.5 µm long, and a patternless rim with 21 - 23 evenly-spaced small triangular protrusions (0.14 - 0.21 µm), gave the scales their distinctive appearance. Surface patterning was similar to the plate scales, with radiating ridges arranged in quadrants and overlying concentric rings, seen on both surfaces (Figs. 4.78, 4.79).

Scale sizes of material from wild samples and cultures from different locations are compared in Table 4.30. Both types of plate scales were slightly smaller from the Tasmanian and Japanese cultured cells in comparison to the wild material from South Africa. There was very little variation in the dimensions of the "eyelash" scales.

Distribution.
This *Chrysochromulina* species was first found in New Zealand coastal waters (Moestrup, 1979). Since then, it has been reported from the Gulf of Elat, South Africa, Japan (Birkhead and Pienaar, 1995a), south-east Australia (Beech, 1983) and again from New Zealand (Rhodes and Burke, 1996).

Toxicity.
In the present study, stationary phase cultures of this *Chrysochromulina* species did not have any detrimental effects on *Artemia* nauplii (see Chapter 7). There are no literature records of any harmful effects associated with this species.

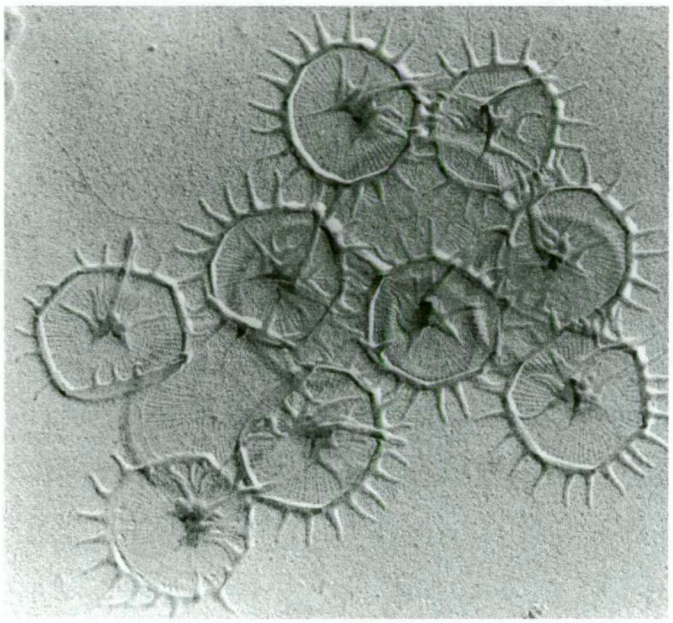


Fig. 4.78: *Chrysochromulina* sp "eyelash" (c. 0.8 μm) and plate scales (0.9 x 0.7 μm); from a Derwent River enrichment culture

(Micrograph no: 4785)

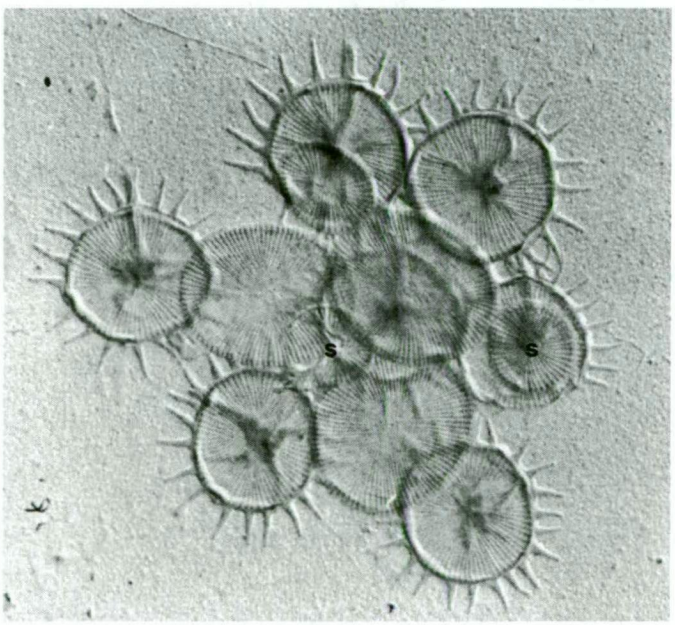


Fig. 4.79: Three different scale types of *Chrysochromulina* sp., including small plate scales (s) (0.5 x 0.4 μm); from the same culture

(Micrograph no: 4795)

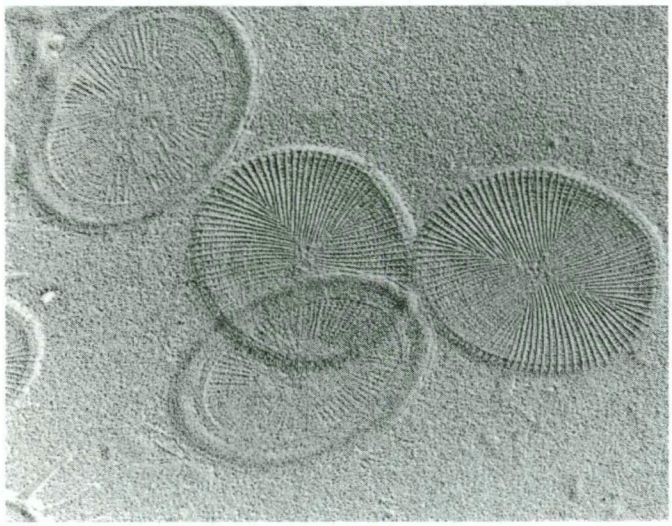


Fig. 4.80: *Chrysochromulina* sp. large plate scales (0.9 x 0.7 μm) - proximal and distal views

(Micrograph no: 4807)

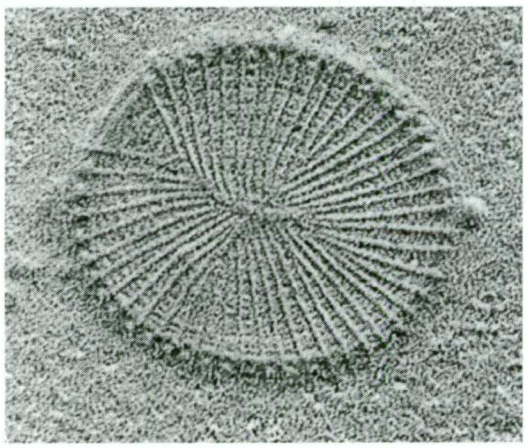


Fig. 4.81: *Chrysochromulina* sp. small plate scale (0.5 x 0.4 μm) - proximal view

(Micrograph no: 4804)

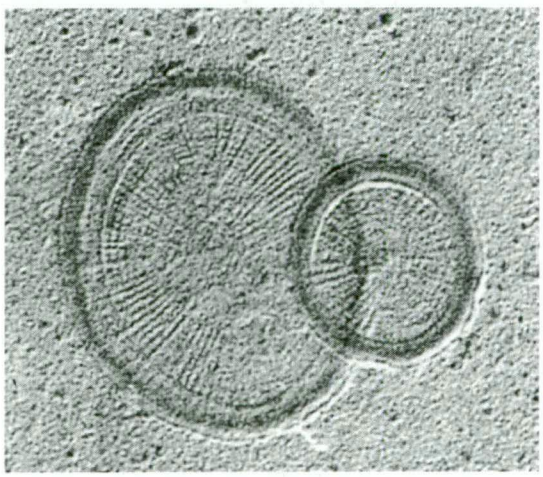


Fig. 4.82: *Chrysochromulina* sp. large (1 μm) and small (0.5 μm) plate scales - distal view

(Micrograph no: 4806)

Table 4.30: Scale sizes of *Chrysochromulina* sp. from different locations.

SOURCE	SMALL SCALES		LARGE SCALES		"EYELASH"SCALES		
	Length (µm)	*Width (µm)*	*Length (µm)*	*Width (µm)*	*Length (µm)*	*Width (µm)*	*Height (µm)*
S. Africa (Pienaar & Bandu, 1984)	0.6	0.5	1.2	0.9	0.7	0.6	0.54
Japan* (Birkhead & Piennar, 1995a)	-	-	0.8 (n=2)	0.7 - 0.8 (n=2)	0.7 - 0.8 (n=2)	0.7 - 0.8 (n=2)	0.46
New Zealand (Moestrup, 1979)	-	-	-	-	0.6 (n=1)	0.6 (n=1)	-
Victoria, Australia (Beech, 1983)	-	-	-	-	0.7 (n=2)	0.6 (n=2)	-
Tasmania, Australia*	0.4 - 0.5 ($\bar{x}$ =0.5; n=7)	0.4 - 0.5 ($\bar{x}$ =0.4; n=7)	0.8 - 1.0 ($\bar{x}$ =0.9; n=26)	0.6 - 0.8 ($\bar{x}$ =0.7; n=26)	0.6 - 0.8 ($\bar{x}$ =0.7; n=40)	0.6 - 0.8 ($\bar{x}$ =0.6; n=40)	0.4 - 0.5 ($\bar{x}$ =0.5; n=9)

* Cultured cells.

Chrysochromulina **sp. 1** **Figs. 4.83 - 4.84**

Present Findings.
Whole cells and scales were found in a Pipeclay Lagoon sample. Scales only were seen in the Southport sample.

This species grew in a GSe/10 enrichment culture derived from a Dru Point sample, but was not established in unialgal culture.

Description.
Cells had an average size of 3 μm (n=4). The two flagella were 8 - 10 μm long (n=6) with thin tips, c. 2 μm. The haptonema was slightly longer than the flagella, c. 15 μm, and appeared to have an irregular sheath (Fig. 4.83).

Scales consisted of a long tapering spine, 1.4 - 1.8 μm long ($\bar{x}$=1.6 μm; n=9), with longitudinal striations. The spine was supported by four struts which extended to the scale rim, dividing the base plate into quadrants. The base plate was round, 0.6 - 0.9 μm in diameter ($\bar{x}$=0.8 μm; n=9), and was patterned with radiating ridges (c. 15 in each quadrant), superimposed on 9 - 10 irregularly-spaced concentric rings (Fig. 4.84).

Scales had a superficial resemblance to those of *C. tenuispina* described from Arctic Canada (Manton, 1978b), in that they had long spines and a strongly patterned base. However, *C. tenuispina* scales have narrower and even longer spines (3 μm), larger base plates (1.3 - 1.6 μm) and a completely different patterning to that observed for the Tasmanian scales.

This material represents a new species of *Chrysochromulina,* which will be formally described elsewhere.

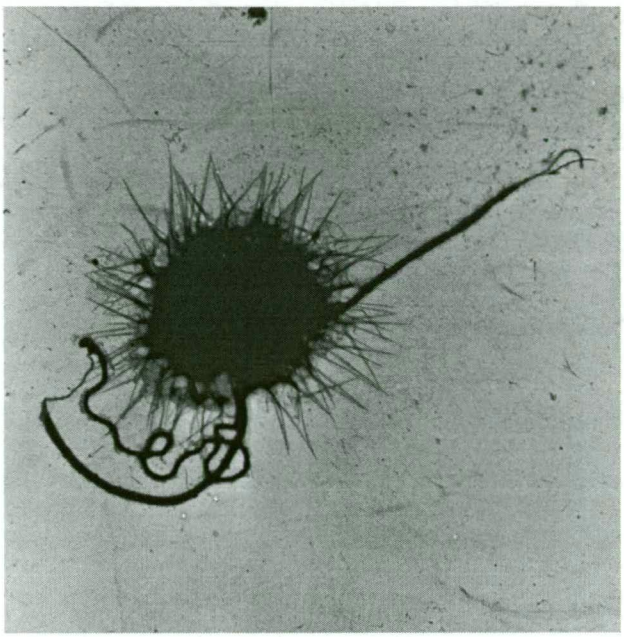


Fig. 4.83: *Chrysochromulina* sp. 1, whole cell (3 μm) showing flagella, haptonema, and spines; from a Derwent enrichment culture

(Micrograph no: 5439)

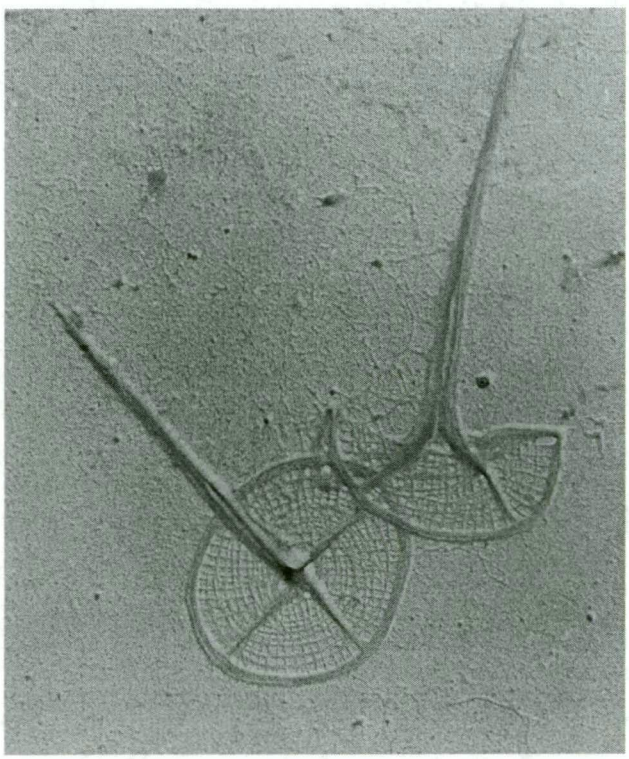


Fig. 4.84: *Chrysochromulina* sp. 1, spine scales (1.5 μm long); from Pipeclay Lagoon

(Micrograph no: 5443)

Chrysochromulina **sp. 2** **Figs. 4.85 - 4.86**

Present Findings.
This cell was found in a sample from Deep Bay.

Description.
The cell, c. 3 µm, had two flagella, 10 and 13 µm, and a coiled haptonema (Fig. 4.85). Small scales had become detached from the cell during sample preparation. These were round to oval, with an average size of 0.36 x 0.28 µm (n=16). They had a very short, "knob-like" spine, less than the scale radius in length, and a raised rim. The scale surface was patterned with c. 30 radiating ridges, arranged in quadrants, and a central cross which was more visible on one surface than the other (Fig. 4.86).

Scales were similar to spined scales of *C. alifera* (Parke et al, 1956) and *C. rotalis* (Eikrem and Throndsen, 1999; Figs. 5 - 7), the latter species being recently described from Danish coastal waters. However there were several differences. *C. alifera* spines are longer than the scale radius, and *C. rotalis* scales have a pattern of concentric fibrils on the proximal surface.

This material represents another new species of *Chrysochromulina,* yet to be formally described.

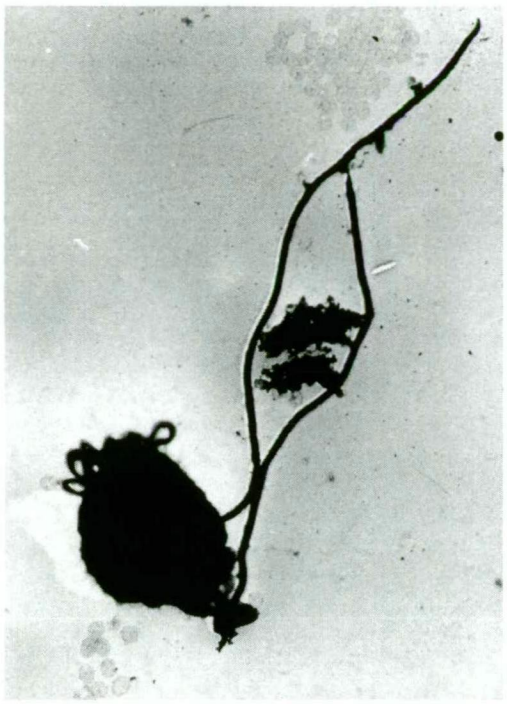


Fig. 4.85: *Chrysochromulina* sp. 2, whole cell (c. 3 μm) showing flagella and coiled haptonema; from Deep Bay

(Micrograph no: 4978)

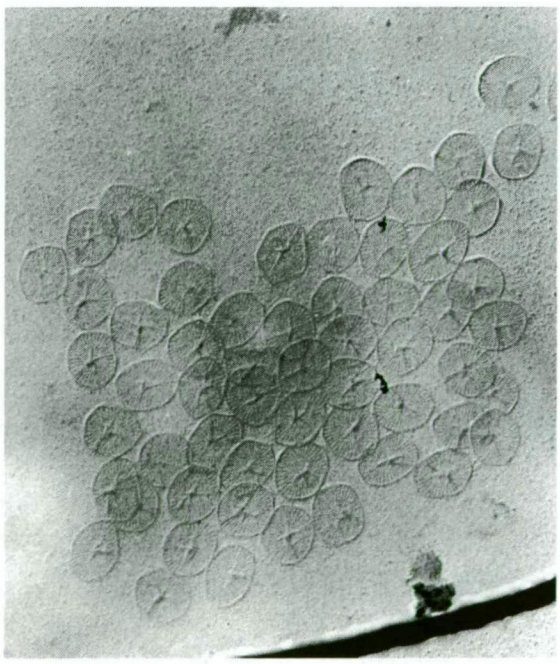


Fig. 4.86: *Chrysochromulina* sp. 2, small scales (0.4 x 0.3 μm) with "knob-like" spine; from Deep Bay

(Micrograph no: 4977)

Chrysochromulina sp. 3 Fig. 4.87

Present Findings.

Two spine scales were found in a Pipeclay Lagoon sample.

Description.

These long spine scales were 5.5 μm, longitudinally striated, with a club-shaped tip (Fig. 4.87a). Spines were attached to the base plate by four struts which did not quite extend to the scale rim. The base plate was 0.7 μm in diameter, and had a well-defined surface pattern of ridges (13 - 15 per quadrant) crossed by irregular fibrils, giving the scale an overall perforated appearance (Fig. 4.87b).

These scales have not been previously illustrated for *Chrysochromulina*, but lack of material (including whole cells) prevented this species from being fully characterised.

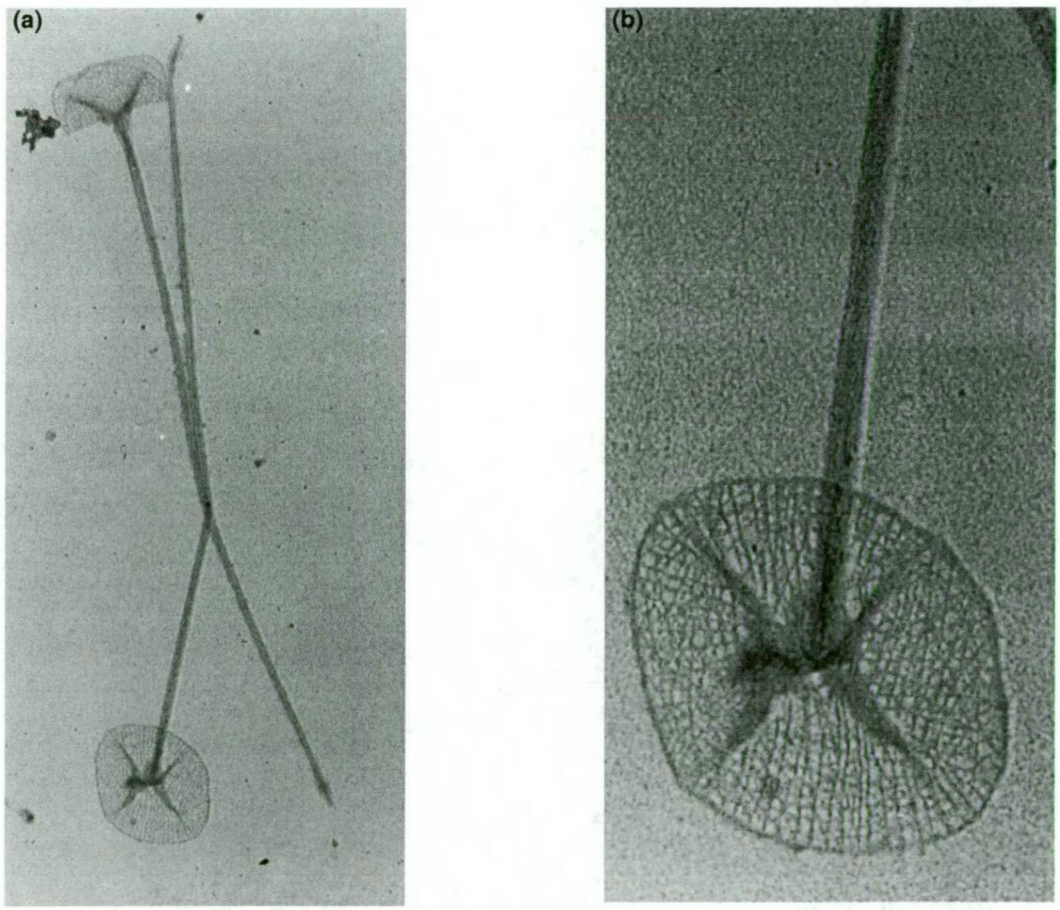


Fig. 4.87: *Chrysochromulina* sp. 3, (a) spine scales (5.5 μm long), (b) detail of base plate patterning (0.7 μm diam.); from Pipeclay Lagoon

(Micrograph no: 5459)

Chrysochromulina sp. 4 **Fig. 4.88**

Present Findings.

Scales were found in samples from the Derwent River, Pipeclay Lagoon and Eaglehawk Neck.

Description.

Two types of scales were observed; spine scales and plate scales (Fig. 4.88).

Spine scales had a tapering spine, 1.1 - 1.4 µm long ($\bar{x}$=1.3 µm; n=8), attached to a base plate by four supporting struts, which did not always extend to the scale rim. The base plate, 1.1 - 1.2 x 0.9 - 1.1 µm ($\bar{x}$=1.2 x 1.0 µm; n=8), was patterned with fine radiating ridges arranged in quadrants (18 - 24 ridges per quadrant). Scales had a patternless raised rim, 0.25 - 0.30 µm high ($\bar{x}$=0.27 µm; n=6).

Plate scales were round to oval, 1.2 - 1.3 x 1.1 - 1.2 µm in diameter ($\bar{x}$=1.3 x 1.2; n=3), with numerous fine radiating ridges (at least 30 per quadrant), seen on both scale surfaces, and a peripheral band, c. 1.0 µm wide (n=1), which was visible only on one surface.

Scales had some similarities to those of *C. brevifilum* (Parke et al, 1955; Birkhead and Pienaar, 1994), but were generally larger (the average spine length was at least twice that of *C. brevifilum* spines), with more radiating ridges on the scale surfaces, and a distinctly higher raised rim on the spine scales.

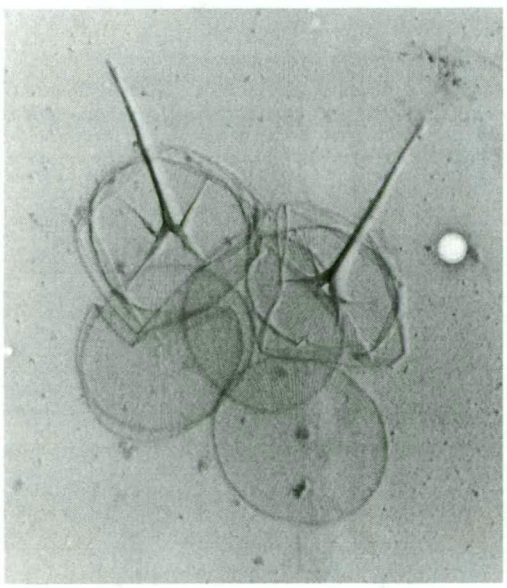


Fig. 4.88: *Chrysochromulina* sp. 4, spine scales (1.0 - 1.4 µm long) with raised rim, and plate scales (1.3 x 1.2 µm); from Eaglehawk Neck

(Micrograph no: 5413)

Chrysochromulina sp. 5 **Fig. 4.89**

Present Findings.

These scales were found in one Derwent River sample.

Description.

There were three scale types: spine scales, large and small plate scales (Fig. 4.89).

Spine scales had a straight central spine, 1.1 - 1.4 μm long ($\bar{x}$=1.3 μm, n=4), supported by four struts which did not extend to the scale edge. The base plate was 1.3 - 1.6 μm in diameter (n=2), patterned with numerous fine radiating ridges, and had a slight raised rim.

Plate scales were of two sizes: 1.4 x 1.3 μm (n=8) and 0.8 x 0.7 μm (n=2). The larger plate scales were more common than the smaller ones. Both scale types had surface patterning of numerous fine radiating ridges arranged in quadrants. The larger plate scales had a peripheral band on one surface, c. 0.15 μm wide.

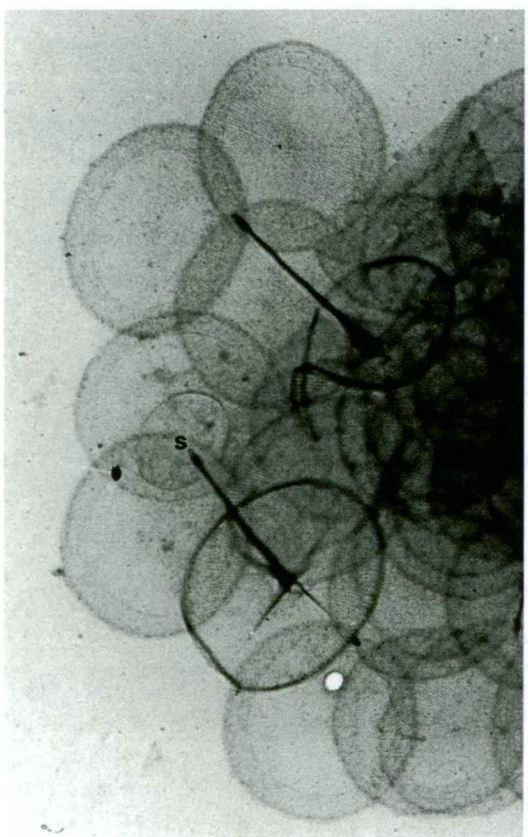


Fig. 4.89: *Chrysochromulina* sp. 5, spine scales (1.3 μm), large plate scales (1.4 x 1.3 μm) and small plate scale (s) (0.8 x 0.7 μm); from the Derwent River

(Micrograph no: 5184)

***Chrysochromulina* sp. 6** **Fig. 4.90**

Present Findings.

Three different scale types were found in Pipeclay Lagoon and Southport samples.

Description.

The first scale type was a small, circular to oval plate scale with an average diameter of 0.6 μm (n=4). It had a raised patternless rim, c. 0.1 μm wide, and a central surface patterning of c. 60 radiating ridges, arranged in quadrants, and superimposed on fine concentric fibrils.

The second type was a larger, oval plate scale, 0.9 x 0.7 μm (n=3), similar in structure to the first type, but with more radiating ridges (c. 70) and a narrower rim, c. 0.05 μm.

The third scale type had a short central spine, 0.13 μm long (n=2), supported by four struts which extended to the scale rim. The base plate, 0.9 x 0.8 μm (n=3), was patterned with radiating ridges (c. 60) and had additional irregularly arranged fibrils. Like the other two types, these scales also had a raised patternless rim, c. 0.05 μm.

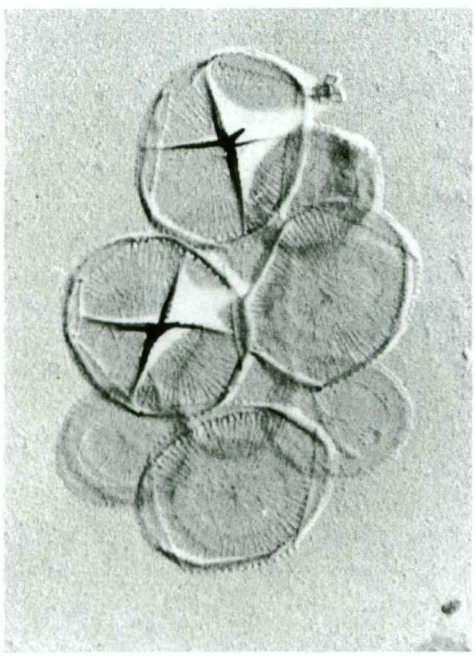


Fig. 4.90: *Chrysochromulina* sp. 6, small (0.6 μm) and large (0.9 x 0.7 μm) plate scales, and spine scales (0.9 x 0.8 μm); from Pipeclay Lagoon

(Micrograph no: 4856)

Chrysochromulina **sp. 7** **Fig. 4.91**

Present Findings.
Scales were found in samples from Honey Moon Bay and Crayfish Point.

Description.
There were two scale types: plate scales and spine scales, both with the typical *Chrysochromulina* pattern of radiating ridges superimposed on fine concentric fibrils (Fig. 4.91). These ridges were evenly-spaced and occasionally slightly curved.

Plate scales were oval, 0.6 x 0.5 μm (n=10), with c. 50 radiating ridges extending to a peripheral band composed of very fine concentric fibrils. The same patterning was seen on both scale surfaces.

Spine scales had a short central spine, 0.1 μm (n=7), supported by struts which again extended to the rim, dividing the scale base into quadrants. They had slightly fewer radiating ridges than the plate scales (c. 9 - 10 per quadrant), but were approximately the same size, 0.6 x 0.5 μm (n=9). Similar patterning was seen on both scale surfaces.

Scales resembled those described by Manton and Oates (1983; Fig. 17c) from the Galapagos Islands. However, there were differences in scale size and patterning. The spine scales were slightly larger (0.7 x 0.5 μm), and plate scales were slightly smaller (0.5 x 0.4 μm) than the Tasmanian material. The patterning on the spine scales consisted of at least twice as many fine radiating ridges, while on the distal surfaces of the plate scales, only concentric fibrils were found.

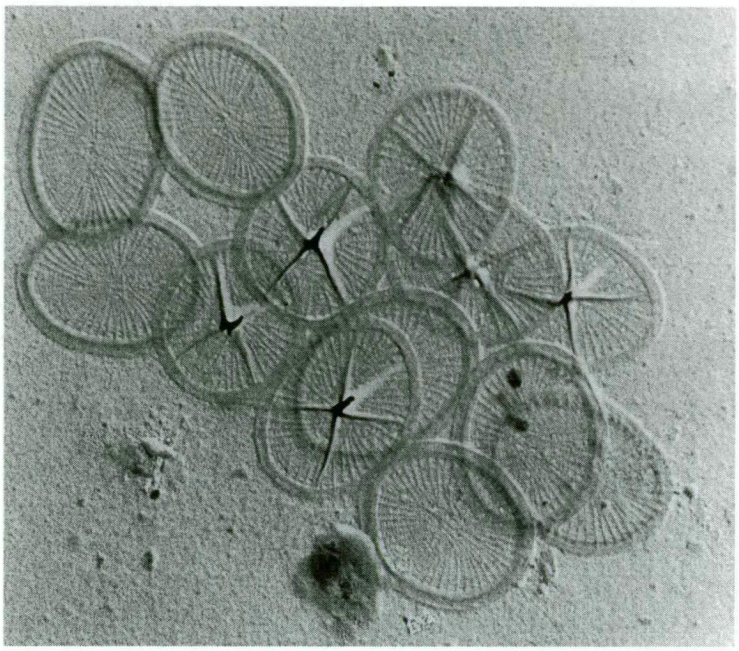


Fig. 4.91: *Chrysochromulina* sp. 7, plate and spine scales (0.6 x 0.5 μm); from Crayfish Point

(Micrograph no: 5505)

Chrysochromulina sp. 8 Fig. 4.92

Present Findings.

Two spine scales were found in a Derwent Estuary sample.

Description.

Scales were circular to oval, 0.7 x 0.6 μm, with 10 - 11 radiating ridges per quadrant, and a raised rim. The central spine, 0.24 - 0.28 μm in length, was supported by four struts which extended to the scale rim, and had a knob-like tip (Fig. 4.92).

These scales had some resemblance to the spine scales of *C. brevifilum* (Parke et al, 1955; Birkhead and Pienaar, 1994). They were the same size, with a similar number of radiating ridges, and a raised rim, but they had shorter and non-tapering spines.

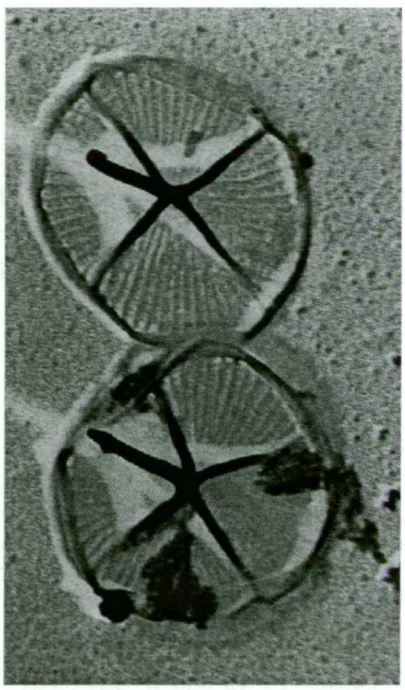


Fig. 4.92: *Chrysochromulina* sp. 8, spine scales (0.7 x 0.6 μm) with central spine (0.24 - 0.28 μm); from Storm Bay

(Micrograph no: 5553)

Chrysochromulina sp. 9 Fig. 4.93

Present Findings.

These scales were found in a Derwent River sample.

Description.

Two types of scales were observed: spine scales and plate scales. Both scale types were round to oval, 1.2 - 1.4 μm (n=4), and patterned with numerous fine radiating ridges. Plate scales had a broad peripheral band, c. 0.1 μm wide. Spine scales had a short blunt spine, 0.2 μm long, supported by four decurrent struts which extended to the scale's raised rim.

Spine scales had a superficial resemblance to the plate scales of *C. vexillifera* (Manton and Oates, 1983) in that they had a distinct central cross, fine radiating ridges and a raised rim, They were also a similar size. However, *C. vexillifera* scales had an oblique cross, rather than a right-angled one, and lacked the short blunt spine seen in this material.

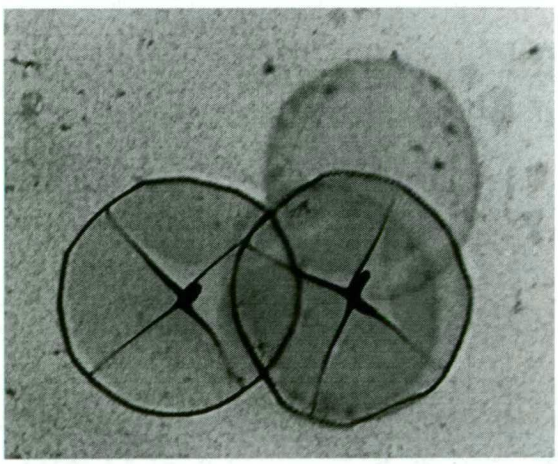


Fig. 4.93: *Chrysochromulina* sp. 9, plate and spine scales (1.2 - 1.4 μm) with central cross; from the Derwent River

(Micrograph no: 5186)

***Chrysochromulina* sp. 10** **Figs. 4.94 - 4.95**

Present Findings.
Three types of scales were found in a Pipeclay Lagoon sample.

Description.
Spine scales resembled those depicted by Manton (1982; Fig. 11) from South Africa. Spines were 0.95 - 1.2 μm in length (n=2), in comparison to 0.8 - 1.1 μm (n=4) in the South African material, and were attached to the base plate by four decurrent struts (Fig. 4.94).

Plate scales were thin with radiating ridges, but details were difficult to distinguish (Fig. 4.94). They were smaller than the South African plate scales, 0.8 x 0.6 μm (n=6) in comparison to 1.1 x 0.8 μm (n=4), and were the most common type of scale.

A third type of scale, not described by Manton, had an unusual "handle-like" central protrusion, and a surface patterning of c. 60 curved radiating ridges superimposed on fine concentric fibrils (Fig. 4.95). These scales were slightly larger than the plate scales, having dimensions of 1.0 x 0.8 μm (n=2).

Scales similar to these have also been found in Norwegian waters (J. Throndsen, pers. comm.), indicating that this undescribed species has a wide distribution.

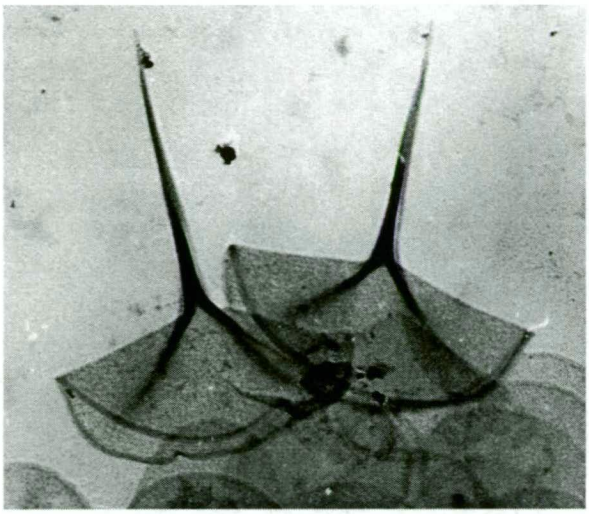


Fig. 4.94: *Chrysochromulina* sp. 10, spine scales (1.0 - 1.2 μm long) and plate scales (0.8 x 0.6 μm); from Pipeclay Lagoon

(Micrograph no: 5211)

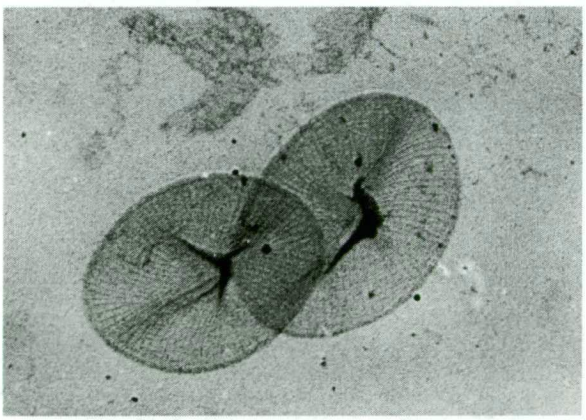


Fig. 4.95: *Chrysochromulina* sp. 10, plate scales (1.0 x 0.8 μm) with "handle-like" protrusion; from Pipeclay Lagoon

(Micrograph no: 5212)

***Chrysochromulina* sp. 11** **Figs. 4.96 - 4.98**

Present Findings.

Scales were found in a Pipeclay Lagoon sample.

Description.

These large plate scales were round to oval, 0.8 - 1.0 μm in size, and very fragile as seen by the folded scales in Fig. 4.96. They had numerous fine radiating ridges, arranged in quadrants, and a faint central oblique cross with one or two small perforations (Figs. 4.97, 4.98). These perforations were not visible on the opposite scale surface, which had a narrow peripheral band composed of faint concentric fibrils (Fig. 4.98).

Scales had some similarities to those described by Leadbeater (1974; Plate 6 D - E) from the Adriatic Sea, and by Beech (1983; Plate 2.12 F) from Port Phillip Bay, Australia. These scales were 0.8 μm in diameter and also had two central perforations. However, in both cases, perforations were larger and more well defined than those seen in the Tasmanian material.

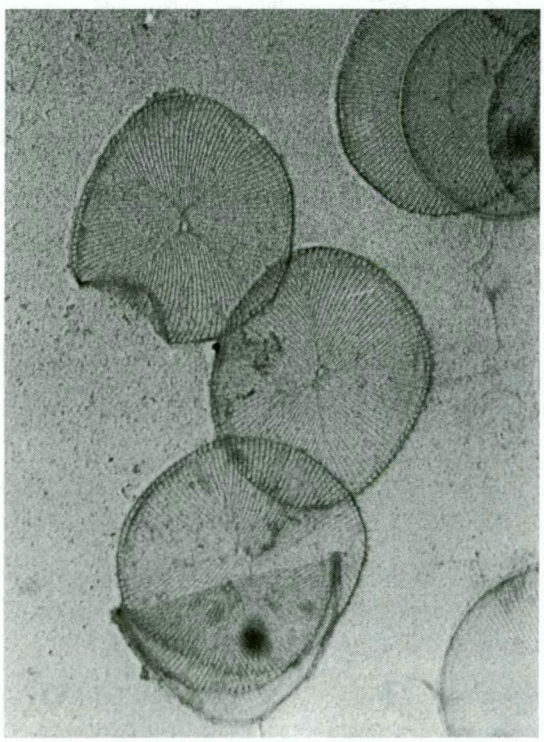


Fig. 4.96: *Chrysochromulina* sp. 11, large plate scales (0.8 - 1.0 μm) showing folded rims; from Pipeclay Lagoon

(Micrograph no: 5199)

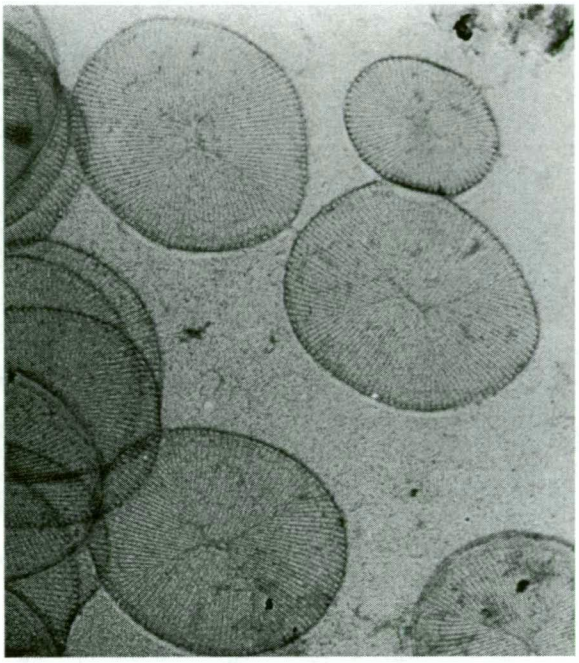


Fig. 4.97: *Chrysochromulina* sp. 11, large plate scales (0.8 - 1.0 μm) with central cross and one or two central perforations; and single small plate scale; from Pipeclay Lagoon

(Micrograph no: 5199)

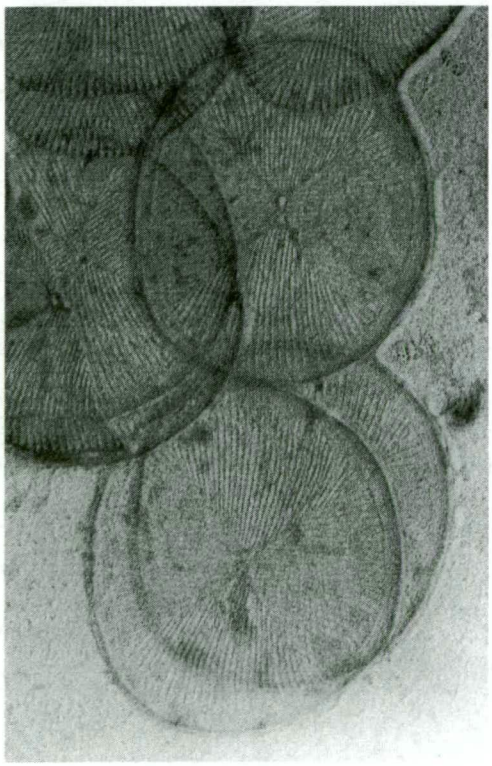


Fig. 4.98: *Chrysochromulina* sp. 11, large plate scales (0.8 - 1.0 μm) - proximal and distal views; from Pipeclay Lagoon

(Micrograph no: 5198)

Chrysochromulina sp. 12 Figs. 4.99 - 4.102

Present Findings.

Scales were found in samples from the Derwent River, Oyster Cove Point and Fleurty Point.

Description.

Scales were round to oval, 0.7 - 0.8 x 0.5 - 0.6 µm in size ($\bar{x}$ = 0.72 x 0.55 µm; n=12) (Fig. 4.99). They had characteristic *Chrysochromulina* scale features, namely radiating ridges (c. 75 - 90) arranged in quadrants, superimposed on numerous fine concentric fibrils on one surface (Fig. 4.101). On the opposite surface, concentric fibrils were clearly visible with radiating ridges more difficult to define, and there was a broad peripheral band (Figs. 4.100, 4.101). The central part of the scale was amorphous. The outer rim was raised, with rectangular perforations corresponding to radiating ridges, and had a thick patternless upper edge (Fig. 4.102).

Scales were very similar to the small plate scales of *C. chiton* and, on initial examination, could easily be identified as belonging to *C. chiton*. However, there were two main differences. The scales described here had a central amorphous area, not seen in *C. chiton* scales, and a concentric fibrillar pattern on one surface, in comparison to the radiating ridges seen on both surfaces of *C. chiton*.

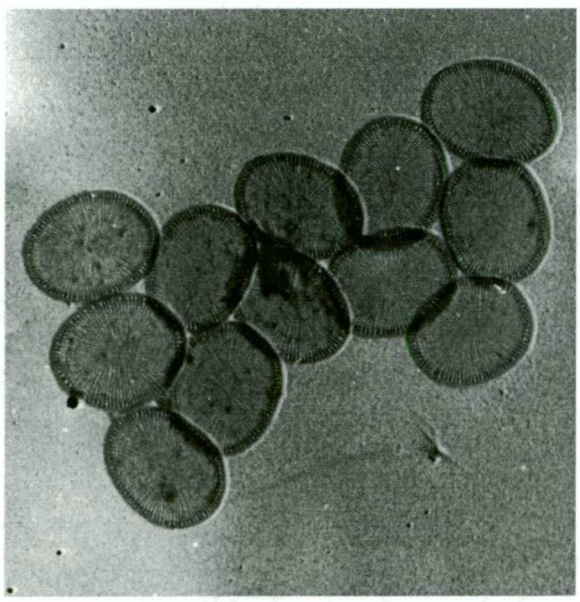


Fig. 4.99: *Chrysochromulina* sp. 12, field of scales; from Oyster Cove Point

(Micrograph no: 4952)

Fig. 4.100: *Chrysochromulina* sp. 12, plate scales (0.7 x 0.5 μm) - distal view; from Fleurty Point

(Micrograph no: 4971)

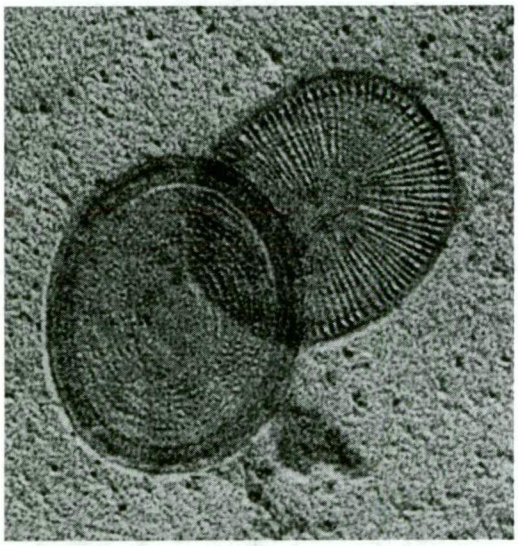


Fig. 4.101: *Chrysochromulina* sp. 12, plate scales (0.7 x 0.5 μm) - proximal and distal views; from Fleurty Point

(Micrograph no: 4971)

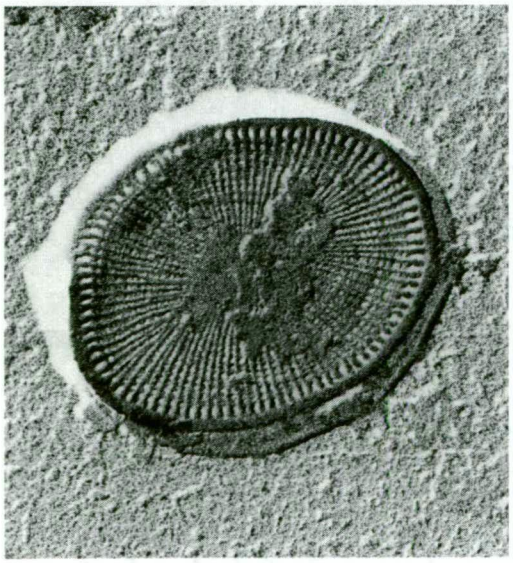


Fig. 4.102: *Chrysochromulina* sp. 12, plate scale showing raised outer rim; from Derwent River

(Micrograph no: 4684)

Chrysochromulina **sp. 13** **Fig. 4.103**

Present Findings.

Two types of scales were seen in a Dru Point sample.

Description.

The larger scale type was oval, 0.8 - 1.0 x 0.6 - 0.7 μm (n=5), with a distinct raised rim. It had a surface patterning of fine radiating ridges (at least 100) arranged in quadrants, and a central elliptical patternless area linked to the rim by four ridges at right angles. The smaller scale type was oval, 0.4 - 0.5 x 0.3 - 0.4 μm (n=6), also with a raised rim. It had a surface patterning of radiating ridges (50 - 60) arranged in quadrants, and a cruciform centre.

Scales had some resemblance to plate scales of *C. mantoniae* (Leadbeater, 1972a), in that the smaller scales were a similar size and the larger plate scales had a distinct raised rim. However, neither scale type of *C. mantoniae* displayed the central cruciform patterning seen in these Tasmanian scales.

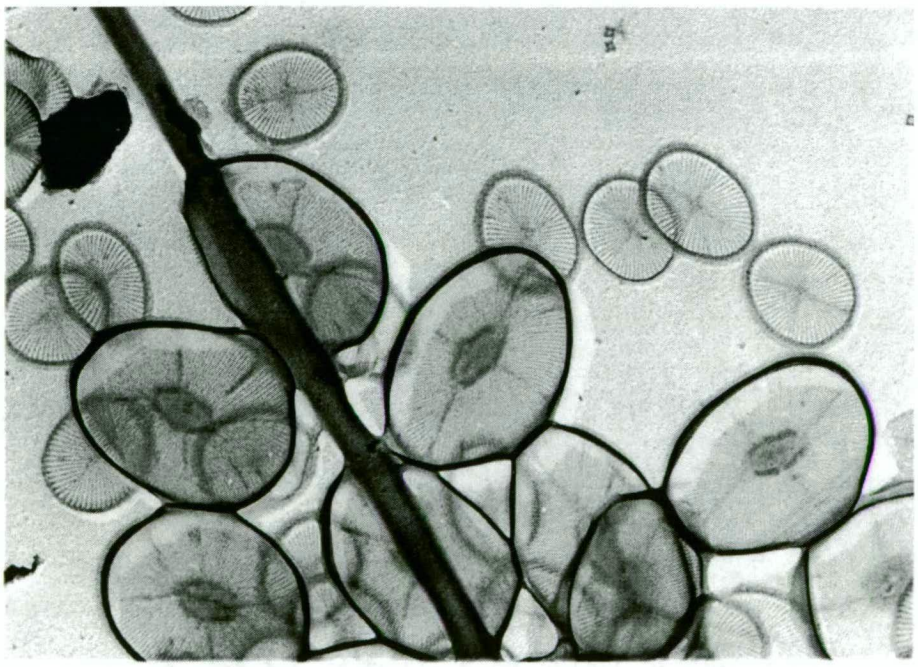


Fig. 4.103: *Chrysochromulina* sp. 13, field of scales; from Dru Point

(Micrograph no: 5171)

***Chrysochromulina* sp. 14** **Figs. 4.104 - 4.105**

Present Findings.
Scales were found in Derwent River and Dru Point samples.

Description.
Three scale types were observed. The first scale type was large and diamond-shaped, 2.0 - 2.8 x 0.6 - 1.6 µm ($\bar{x}$=2.2 x 1.1 µm; n=4), with a surface patterning of numerous fine radiating ridges and a raised patterned rim (Fig. 4.105).

The second type was oval, 1.3 - 1.7 x 0.8 - 1.1 µm ($\bar{x}$=1.5 x 0.9 µm; n=6), with numerous fine radiating ridges on one surface and concentric fibrils on the other (Fig. 4.104). This scale had a rim consisting of two rows of perforations, and was the most common scale type.

The third type was the least frequently observed, and was long and elliptical, 1.3 x 0.3 µm (n=2), with irregularly arranged perforations and an ornate rim (Fig. 4.104). It was difficult to determine further structural details from the material available.

The first two scale types had some resemblance to *C. parkae* scales, in that they were similar in size and shape. However, the surface patterning of *C. parkae* scales was very different, having crescent-shape fibrils not seen in this material (Green and Leadbeater, 1972). The large diamond-shaped scales of *C. parkae* lacked perforated rims, while the smaller plate scales had rims with only one row of perforations.

The third scale type had a superficial resemblance to the long elliptical scales of *C. polylepis* (Paasche et al, 1990), but lacked the spined projections at one or both ends, instead having an ornate perforated structure. These scales were also considerably smaller in size than those of *C. polylepis.*

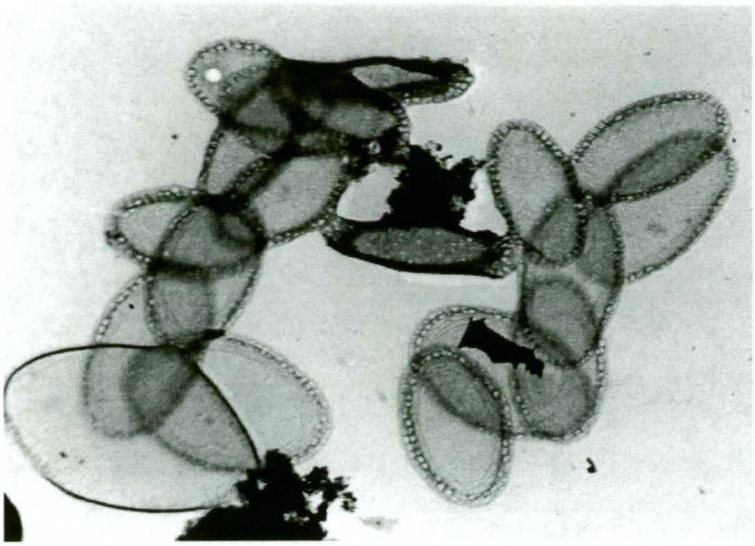


Fig. 4.104: *Chrysochromulina* sp. 14, three scale types, including oval scales (1.5 x 0.9 μm) and long elliptical scales (1.3 x 0.3 μm), and one large scale (2.0 x 0.8 μm); from Dru Point

(Micrograph no: 5175)

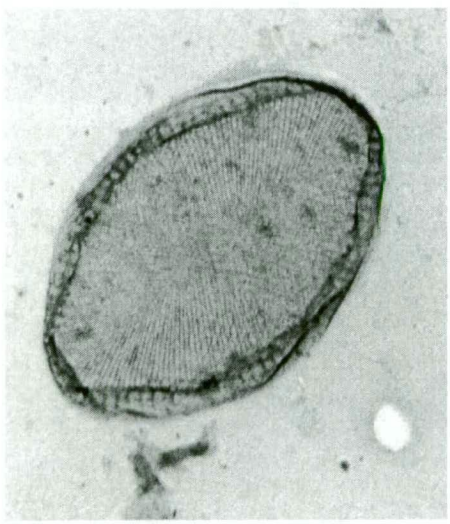


Fig. 4.105: *Chrysochromulina* sp. 14, large scale (2.0 x 1.1 μm); from Dru Point

(Micrograph no: 5175)

Chrysochromulina sp. 15 Fig. 4.106

Present Findings.

Scales were found in a Pipeclay Lagoon sample.

Description.

Scales were oval, 1.0 - 1.2 x 0.75 - 0.90 μm (n=4), and had a central irregular area and a raised rim. They were patterned with rectangular perforations, arranged in 9 - 10 concentric circles, with 70 perforations in the outer ring, gradually decreasing in number towards the scale centre.

Three other *Chrysochromulina* species have scales patterned with concentric perforations, namely *C. leadbeateri*, *C. throndsenii* and *C. polylepis* (Edvardsen et al, 1996; Eikrem, 1996; Eikrem and Throndsen, 1998), but none of these species had scales with a central projection. The scales described here were larger than those of both *C. throndsenii* and *C. leadbeateri* and had more numerous perforations. They were slightly smaller in size and had fewer outer perforations than the large oval scales of *C. polylepis*. In addition, the outer perforations of *C. polylepis* are larger and distinctly rectangular in comparison to those observed in this material.

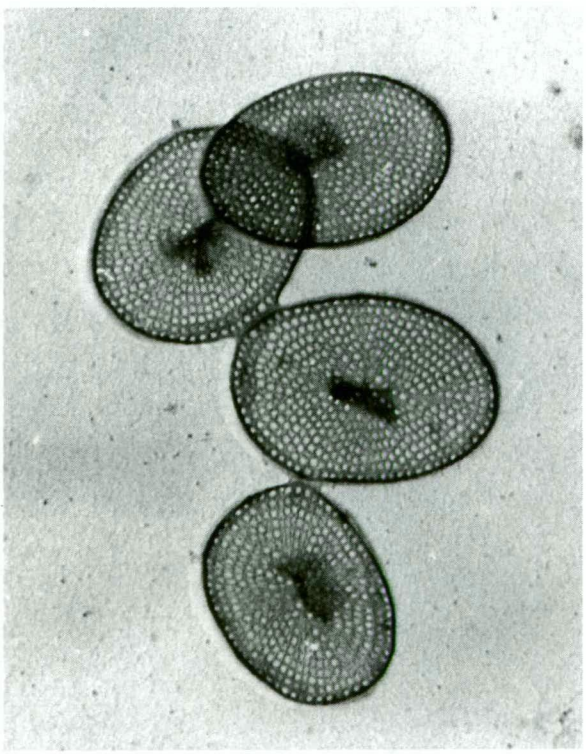


Fig. 4.106: *Chrysochromulina* sp. 15, field of scales (1.2 x 0.9 μm); from Pipeclay Lagoon

(Micrograph no: 5219)

Chrysochromulina **sp. 16** **Figs. 4.107 - 4.108**

Present Findings.
Two oval scale types were found in a Derwent River sample and in a Pipeclay Lagoon sample.

Description.
The smaller scale type, 0.4 x 0.3 µm (n= 8) had a central cross and a perforated pattern composed of 30 - 35 radiating ridges crossing a spiralling rib (Figs. 4.107, 4.108). In these aspects, it was similar to scales of *C. throndsenii* described by Eikrem (1996; Figs. 3 - 4) from Norway. However, *C. throndsenii* scales are smaller (0.30 - 0.35 µm in diameter), have fewer radiating ribs (c. 25) and typically have two spiralling ribs making up the concentric pattern, (although scales with one spiralling rib have been recorded). Also, some *C. throndsenii* scales have an upright rim, which was not seen on any of the Tasmanian scales.

These scales differed from those of *C. leadbeateri* and *C. polylepis* (Edvardsen et al, 1996; Eikrem and Throndsen, 1998), which also have a perforated surface pattern, in that they had radiating ridges crossing a spiralling rib, rather than concentric fibrils.

The larger scale type, 0.7 x 0.5 µm (n=5), had a distinct rim and a faint pattern of small concentric perforations. Five to eight larger perforations were consistently seen in the centre of the scale (Figs. 4.107, 4.108).

This scale type was almost twice the size of *C. leadbeateri* or *C. throndsenii* scales and lacked the obvious perforated pattterning of these scales, and of the similar-sized oval scales of *C. polylepis*. However, *C. polylepis* also has an "alternate" form, described by Paasche et al (1990), which includes three scale types. Of these, the large plate scales have an faint perforated pattern not unlike the one seen here, and a distinct rim, but they are over twice the size of the Tasmanian scales.

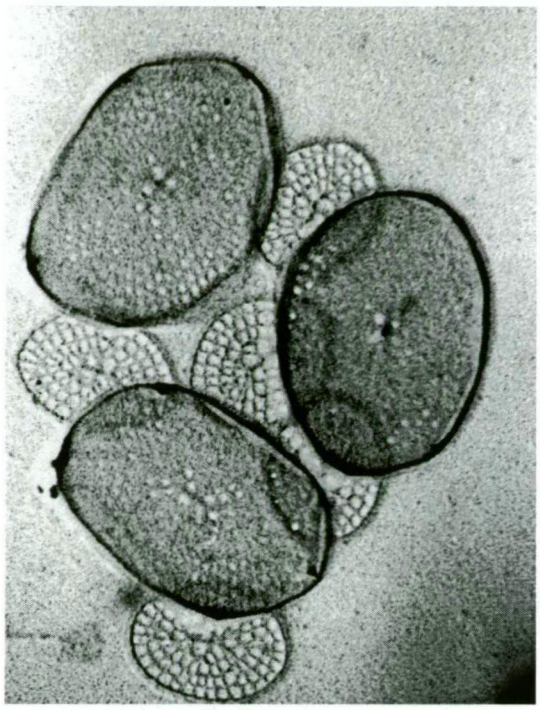


Fig. 4.107: *Chrysochromulina* sp. 16, field of scales; from the Derwent River

(Micrograph no: 5221)

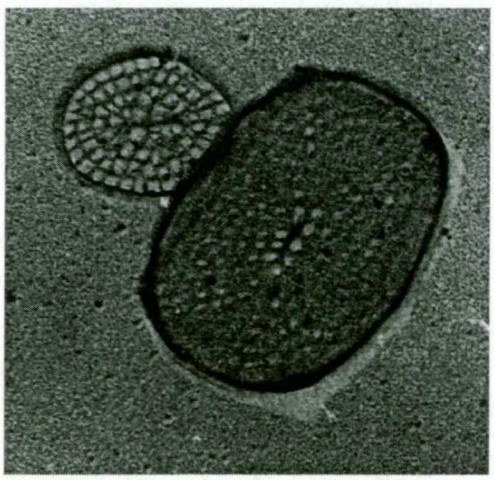


Fig. 4.108: *Chrysochromulina* sp. 16, large scale (0.7 x 0.5 μm) with raised rim, and small perforated scale (0.4 x 0.3 μm) with spiralling rib; from the Derwent River

(Micrograph no: 5228)

Chrysochromulina **sp. 17** **Fig. 4.109**

Present Findings.

Two scale types were observed from Pipeclay Lagoon and Honey Moon Bay samples.

Description.

The first scale type was round to oval, 1.3 - 1.4 x 1.0 μm (n=2), with fine radiating ridges in quadrants (c. 30 ridges per quadrant), and a central cross. Scales were rimmed and had a short central knob on one surface.

The second scale type was "box-like" with a square base, 1.2 x 1.1 μm (n=2). It had a central square concentric pattern, superimposed on traversing ridges which formed quadrants, as well as a wide patternless rim, c. 0.3 μm. A short diagonal line was consistently seen in the centre of the scale.

A similar square concentric pattern was observed on an oval unidentified *Chrysochromulina* scale described from Victorian coastal waters (Beech, 1983; Plate 2.12 E).

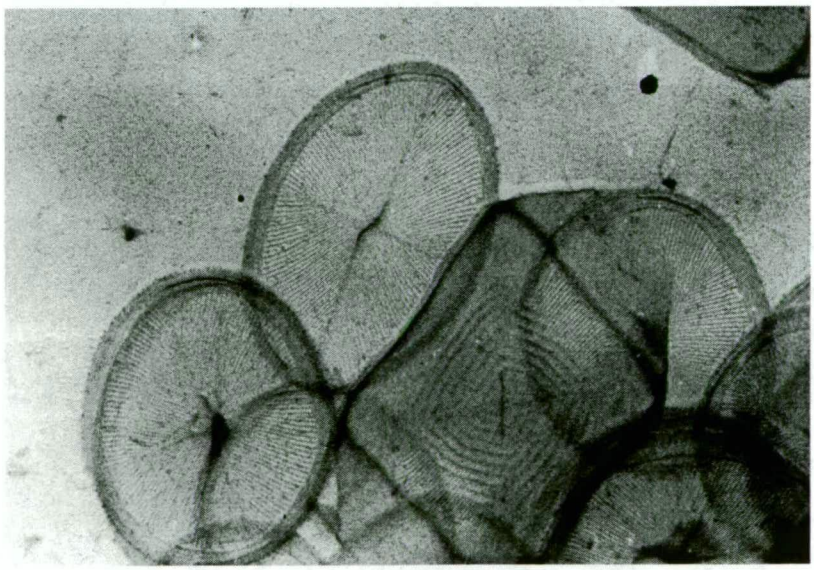


Fig. 4.109: *Chrysochromulina* sp. 17, oval scales (1.3 - 1.4 x 1.0 μm) and a large "box-like" scale (1.2 x 1.1 μm); from Pipeclay Lagoon

(Micrograph no: 5207)

Chrysochromulina sp. 18 Fig. 4.110

Present Findings.

This scale was found in a Derwent River sample.

Description.

The scale, 1.0 x 0.8 μm, had a typical *Chrysochromulina* pattern of numerous radiating ridges arranged in quadrants and superimposed on concentric fibrils, as well as a raised rim and a central projection, c. 0.15 μm long.

This scale was similar to the smaller scales of *C. birgeri*, described by Hällfors and Thomsen (1979; Fig. 8) from Finland. *C. birgeri* scales have a characteristic "horn-like" central projection on the distal surface. Although details of the central projection in the Tasmanian scale were unclear, its length was the same as that of *C. birgeri* scales. Other similarities included scale size, a round to oval shape and a distinct rim. However, due to lack of structural detail for the central projection, this scale could not be confirmed as belonging to *C. birgeri.*

C. birgeri has been known to bloom under ice in the Gulf of Finland (Hällfors and Thomsen, 1979) and, in 1996, a *C. birgeri* bloom in Nova Scotia resulted in fish mortality (Edvardsen and Paasche, 1998).

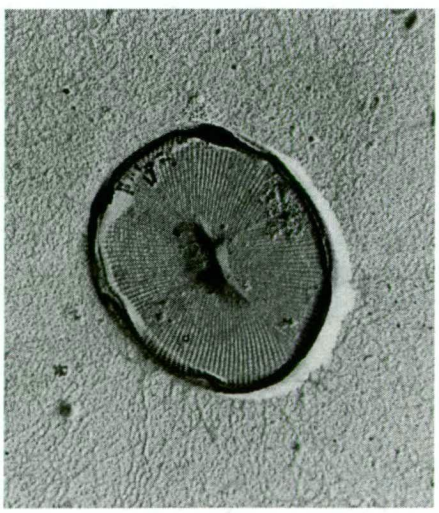


Fig. 4.110: *Chrysochromulina* sp. 18, oval scale (1.0 x 0.8 μm) with central projection; from the Derwent River

(Micrograph no: 5331)

***Chrysochromulina* sp. 19** **Figs. 4.111 - 4.112**

Present Findings.

This scale was found in the Eaglehawk Neck sample.

Description.

The scale was oval, 1.2 x 1.0 μm, with a small central triangular spine, supported by four short struts, and had a raised rim. It had a well-defined radial surface pattern, composed of non-concentric oval fibrils overlying evenly-spaced radiating ridges (Fig. 4.111). A similar pattern was seen on the distal scale surface of *C. tenuisquama*, described by Estep et al (1984; Figs. 20 - 21) from the Sargasso Sea and the south coast of Florida. However, *C. tenuisquama* scales were half the size of the scale seen here, and they did not have a central spine.

Another scale type with a radial pattern has been described by Beech (1983; Plate 12 D) from Port Phillip Bay, Victoria. This scale type was also found in a Port Phillip Bay sample which I collected in 1997 and is included here for comparison (Fig. 4.112). Surface patterning consisted of 16 - 18 well-defined concentric fibrils overlying numerous, evenly-spaced radiating ridges. The scale had an upright patterned rim, c. 0.12 μm high, and a central triangular projection, c. 0.2 μm.

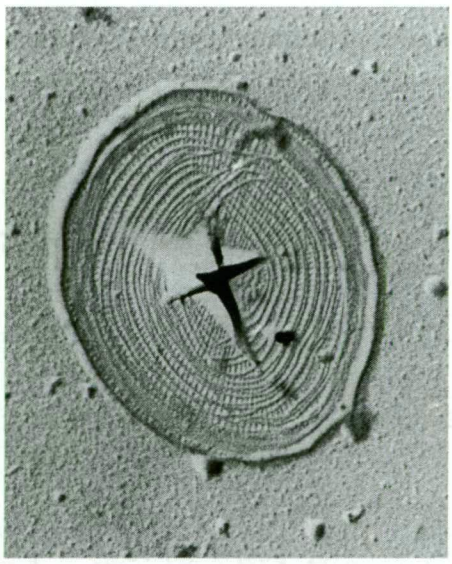


Fig. 4.111: *Chrysochromulina* sp. 19, scale (1.2 x 1.0 μm) with central spine and radial pattern; from the Derwent River

(Micrograph no: 5406)

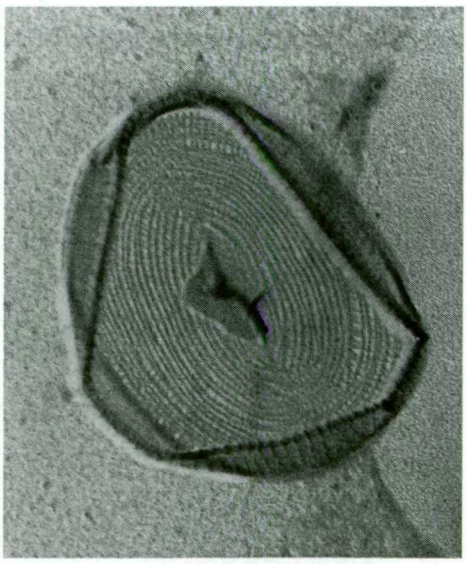


Fig. 4.112: *Chrysochromulina* sp., scale (1.1 x 0.8 μm) with central projection and radial pattern; from Port Phillip Bay, Victoria

(Micrograph no: 5582)

***Corymbellus aureus* Green** **Fig. 4.113**

Micrographs: Green, 1976; Plate 1 K - L.
Moestrup, 1979; Fig. 27.
Hallegraeff, 1983; Fig. 19.
Estep et al, 1984; Figs. 28 - 29.
Hoepffner and Haas, 1990; Fig. 5.
Thomsen et al, 1994; Fig. 16.

Present Findings.
Scales were found in samples from Dru Point, Crayfish Point and Honeymoon Bay. A cell retaining scales, but lacking flagella and haptonema, was seen in a Pipeclay Lagoon sample.

Description.
Corymbellus aureus is unusual, as it is the only motile colonial member of the Prymnesiophyceae (Green, 1976). Unfortunately, no live colonies were seen in this survey.

C. aureus has two types of scales, oval spined scales and smaller plate scales, but only the spined scales were observed in the Tasmanian material (Fig. 4.113). Scales were 0.29 - 0.37 x 0.20 - 0.27 μm in size ($\bar{x}$=0.32 x 0.23; n=15). Each scale had a central short spine, consisting of four struts bridging a central pore, and a distinct upright rim. The base of the scale was patterned with numerous fine radiating ridges.

These scales were similar in size and structure to those of the type material (Green, 1976) and those reported from other studies (Table 4.31). Estep et al (1984) recorded scales with two central pores; however, this may have been due the "bridging effect" of the central spine. Fig. 4.113 also shows scales which seem to have two central pores.

Distribution.

C. aureus was orginally reported from the English Channel (Green, 1976) and has since been found in coastal and oceanic waters of both hemispheres, including New Zealand coastal waters (Moestrup, 1979), the East Australian Current (Hallegraeff, 1983), the North Atlantic and North Pacific Oceans (Estep et al, 1984; Hoepffner and Haas, 1990), a Canadian fjord (Smith and Hobson, 1994), Californian coastal waters and the Beagle Channel at the southern tip of South America (Thomsen et al, 1994).

C. aureus has the potential to form blooms, and in 1983, a spring bloom of 9 x 10^5 cells mL^{-1} occured in the North Sea (Gieskes and Kraay, 1986).

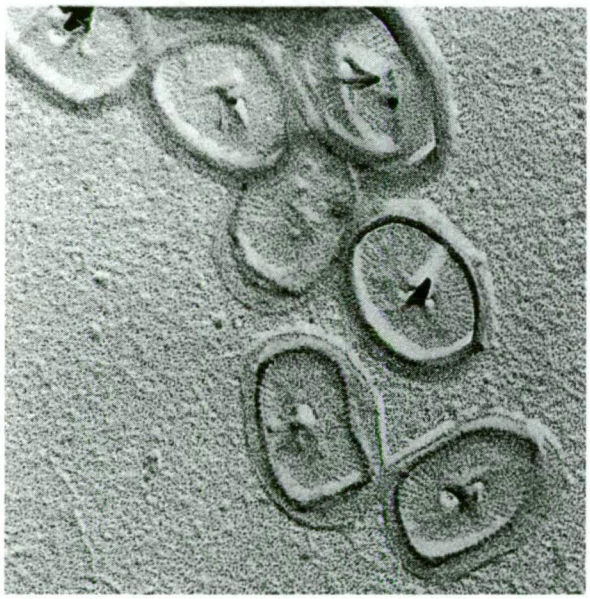


Fig. 4.113: *Corymbellus aureus* scales (0.3 x 0.2 µm) showing short central spine; from Honey Moon Bay

(Micrograph no: 5357)

Table 4.31: Size of *Corymbellus aureus* spined scales from different locations.

SOURCE	SPINED SCALES	
	Length (μm)	*Width (μm)*
English Channel *(type)* (Green, 1976)	0.30 - 0.35	0.21 - 0.25
New Zealand (Moestrup, 1979)	0.35 - 0.37	0.25 - 0.27
East Australian Current (Hallegraeff, 1983)	0.30 - 0.38	0.26 - 0.30
North Atlantic (Estep et al, 1984)	0.33 - 0.43 ($\bar{x}$ =0.40, n=4)	0.22 - 0.30 ($\bar{x}$ =0.28, n=4)
North Pacific (Hoepffner & Haas, 1990)	0.25 - 0.28 ($\bar{x}$ =0.26, n=4)	0.21 - 0.22 ($\bar{x}$ =0.22, n=4)
Tasmania, Australia	0.29 - 0.37 ($\bar{x}$ =0.32, n=15)	0.20 - 0.27 ($\bar{x}$ =0.23, n=15)

***Imantonia rotunda* (Reynolds) Green et Pienaar** **Fig. 4.114**

Micrographs: Reynolds, 1974; Figs. 5 and 7.
Green and Pienaar, 1977; Plate 3 B - C.
Beech, 1983; Plate 2.14 A - B.
Hallegraeff, 1983; Fig. 20.
Thomsen, 1986; Figs. 16 - 17.

Present Findings.

Imantonia rotunda was commonly found in this survey. Whole cells, as well as scales, were frequently observed in samples from most areas, both in wild material and in enrichment cultures.

I. rotunda was seen in samples from the Derwent River, the D'Entrecastreaux Channel (Dru Point, Oyster Cove Point and Southport), Roches Beach, Pipeclay Lagoon, Pirates Bay and Honey Moon Bay. It grew easily in enrichment cultures (GSe, GSe/2, GSe/10, K/2 and ML media) and was found in cultures established from the Derwent River, the D'Entrecasteaux Channel (Dru Point, Oyster Cove Point and Fleurty Point), Port Huon, Pipeclay Lagoon, Pirates Bay, Honey Moon Bay and Coles Bay.

Description.

Cells were about 2 - 3 μm diameter had two equal flagella, c. 4 μm in length (Fig. 4.114). There was no evidence of a haptonema as such; however, a short stub (0.7 μm) was observed between the flagella on one cell (Fig. 4.114). This has been described as a "proboscis" containing endoplasmic reticulum (Green and Pienaar, 1977). Cell dimensions were in the lower size range in comparison to the emended type description by Green and Pienaar, (1977)

I. rotunda has characteristic "bicycle-wheel" scales. Scales observed were circular to oval with 20 - 24 evenly-spaced ridges (or "spokes"!) radiating from the centre and extending to the scale margins; there was a faint underlying pattern of concentric rings. There were two types of scales, both c. 0.5 - 0.7 μm, differentiated by the presence of a broad upright rim, 0.10 - 0.15 μm high ($\bar{x}$=0.13 μm; n=7) (Fig. 4.115). The rimmed scales were not as common as those without rims, and they were not found on all cells, which agreed with previous observations (Green and Pienaar, 1977; Hallegraeff, 1983).

I. rotunda scales were consistent in structure and size, regardless of location (cold, temperate or subtropical waters) or sample type (wild or cultured material) (Table 4.32).

Distribution.

I. rotunda has been recognised from a wide variety of locations and is common in both inshore and offshore waters. It has been recorded mostly from colder waters of the northern hemisphere, including: the Arctic, UK (Reynolds, 1974), USA (Green and Pienaar, 1977; Anderson et al, 1991), Denmark (Thomsen, 1986) and Canada (Anderson et al, 1991; Smith and Hobson, 1994). It has also been reported from temperate and subtropical waters of both hemispheres, including those of: Mexico (Anderson et al, 1991), the Canary Islands, South Africa (Reynolds, 1974) and Australia (Beech, 1983; Hallegraeff, 1983).

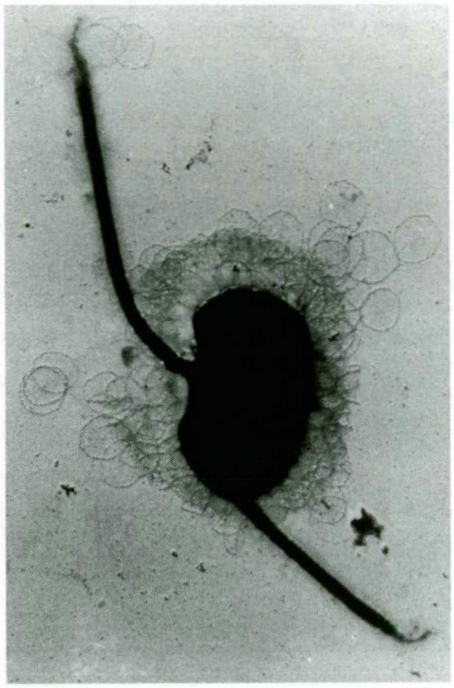


Fig. 4.114: *Imantonia rotunda* cell (c. 3 μm); from Coles Bay

(Micrograph no: 5428)

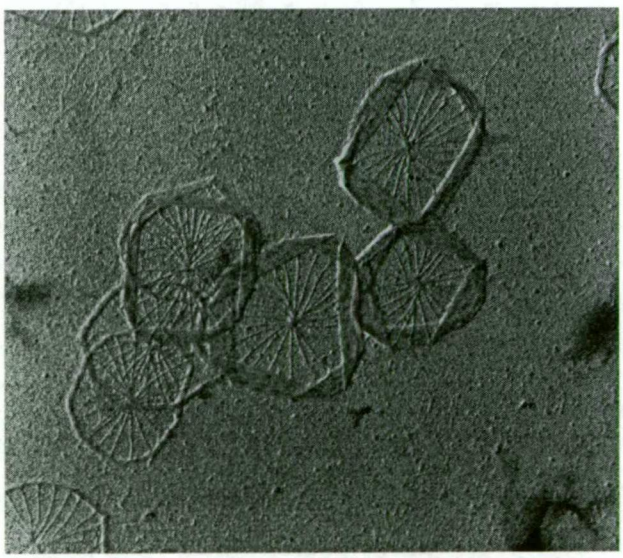


Fig. 4.115: *Imantonia rotunda* "bicycle-wheel" scales, (0.5 - 0.7 μm), with and without rims; from a Derwent enrichment culture

(Micrograph no: 4908)

Table 4.32: *Imantonia rotunda* scales from different locations.

SOURCE		RIMLESS SCALES		RIMMED SCALES		
		Dimensions (μm)	*No. of Ridges*	*Dimensions (μm)*	*Rim Width (μm)*	*No. of Ridges*
Arctic *(type)* (Reynolds, 1974)	Cultured cells only	0.4 - 0.5 x 0.3 - 0.4 ($\bar{x}$ =0.4, n=3)	17 - 20	0.6 x 0.4 - 0.5 ($\bar{x}$ =0.6 x 0.5, n=2)	0.02 - 0.10	17 - 20
UK and USA (Green & Pienaar, 1977)	Wild material & cultured cells	0.45 - 0.68	16 - 23	0.72 - 0.80	0.10 (n=3)	22 - 23 (n=2)
Denmark (Thomsen, 1986)	Wild material only	0.6 - 0.7 x 0.5 - 0.6 ($\bar{x}$ =0.6, n=3)	16 - 18 (n=3)	-	-	-
East Australian Current, Australia (Hallegraeff, 1983)	Wild material & cultured cells	0.5 - 0.7	18 - 24	0.5 - 0.7	0.11 (n=1)	18 - 24
Victoria, Australia (Beech, 1983)	Wild material only	0.5 - 0.7	22	0.5 - 0.7	ND	21
Tasmania, Australia	Wild material & cultured cells	0.5 - 0.8 x 0.5 - 0.7 ($\bar{x}$ =0.6, n=29)	22 - 24 (n=27)	0.6 - 0.8 x 0.5 - 0.7 ($\bar{x}$ = 0.70 x 0.6, n=9)	0.10 - 0.15 ($\bar{x}$ =0.13, n=7)	20 - 21 (n=7)

ND = Not determined

***Phaeocystis globosa* Scherffel** **Figs . 4.116 - 4.119**

Micrographs: Parke et al, 1971; Figs. 11 - 12, 16 - 19.
Moestrup, 1979; Fig. 35.
Hallegraeff, 1983; Fig. 21.

Present Findings.

Phaeocystis globosa was found at most of the sites in this survey: the Derwent River, Crayfish Point, Dru Point, Fleurty Point, Oyster Cove Point, Port Huon, Southport, Pipeclay Lagoon, Roches Beach, Honey Moon Bay, Little Swanport and Pirates Bay.

P. globosa also grew in GSe enrichment cultures from the Derwent River, Maria Island and Pirates Bay, and is currently maintained as a unialgal isolate (CS-495) in the CSIRO Culture Collection of Living Microalgae (at 15°C, under standard growth conditions). Both colonies and free-living cells are observed in culture.

Description.

The key feature used to identify *P. globosa* in this study was the presence of pentagonal star-like structures, c. 1.0 µm diameter (n=4), produced by the motile free-living cell (Figs. 4.117, 4.118). Once released from the cell, the threads forming these star-like structures coiled about the cell (Fig. 4.116), before being fully discharged. Such threads were much longer than the cell and had a finely tapering tip (Fig. 4.118).

These pentagonal star-like structures structures are also associated with two other species of *Phaeocystis*, *P. antarctica* and *P. pouchetii.* However, both these species are usually found in polar and subpolar regions with optimal growth temperatures of 4.5°C and 8°C respectively (Baumann et al, 1994). Thus, features described for *P. pouchetii* by Parke et al (1971), Moestrup (1979) and Hallegraeff (1983) have been attributed to *P. globosa* (Medlin et al, 1994).

Scales of *P. globosa* were only seen on motile cells. There were two scale types: larger oval scales, 0.22 - 0.24 x 0.18 - 0.19 µm (n=3), and smaller circular scales, 0.13 µm in diameter (n=3), both with a surface pattern of radiating ridges (Fig. 4.119).

The scales of *P. pouchetii* and *P. antarctica* have not yet been fully characterised (Medlin et al, 1994).

Colonies of *P. globosa* found in enrichment cultures were beautifully spherical, with single cells distributed evenly along the periphery of the colony. This is similar to the pattern of cell distribution found in *P. antarctica* colonies. In contrast, colonies of *P. pouchetii* are round to lobed with groups of cells spread throughout the mucilaginous matrix (Medlin et al, 1994).

Distribution.

Due to the confusion in identifying specific species of *Phaeocystis*, it is difficult to determine the specific distribution of *P. globosa* from literature reports. Temperature tolerance work done by Baumann et al (1994) showed that the optimum growth temperature for *P. globosa* was 16°C with a growth range of 4 - 22°C, indicating that *P. globosa* will be found in temperate to warm waters of both hemispheres.

Toxicity.

Phaeocystis blooms have had negative impacts on fishing, aquaculture and tourism, usually as a result of producing dense foams. These blooms are often avoided by fish and appear to be detrimental to the growth and reproduction of shellfish and macrozooplankton (Davidson and Marchant, 1992).

Phaeocystis may also form toxins responsible for fish kills (Stabell et al, 1999). In the present work, *P. globosa* was tested for toxicity using *Artemia* nauplii; no nauplii mortalities were observed after 24 hours (see Chapter 7).

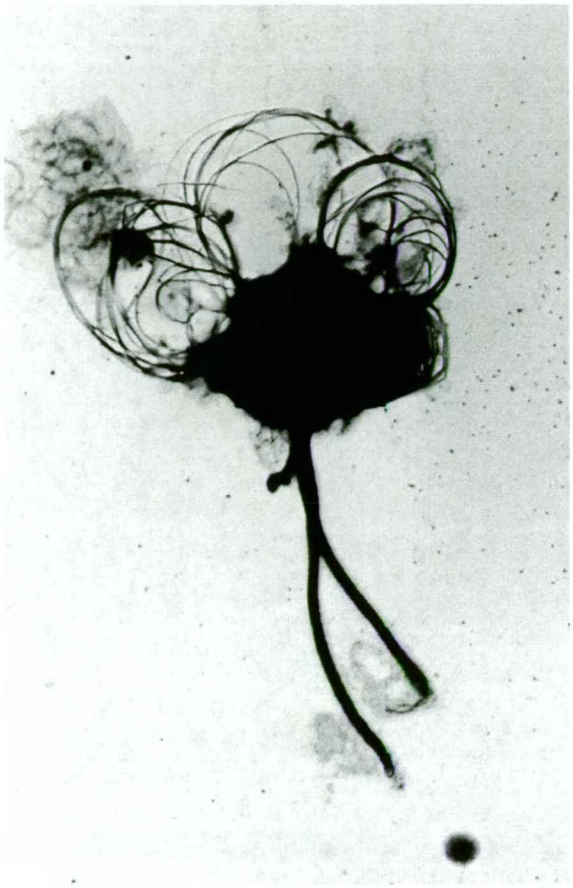


Fig. 4.116: *P. globosa* cell (0.5 - 0.7 μm), with star-like structures coiled about the cell; from a Derwent enrichment culture

(Micrograph no: 5593)

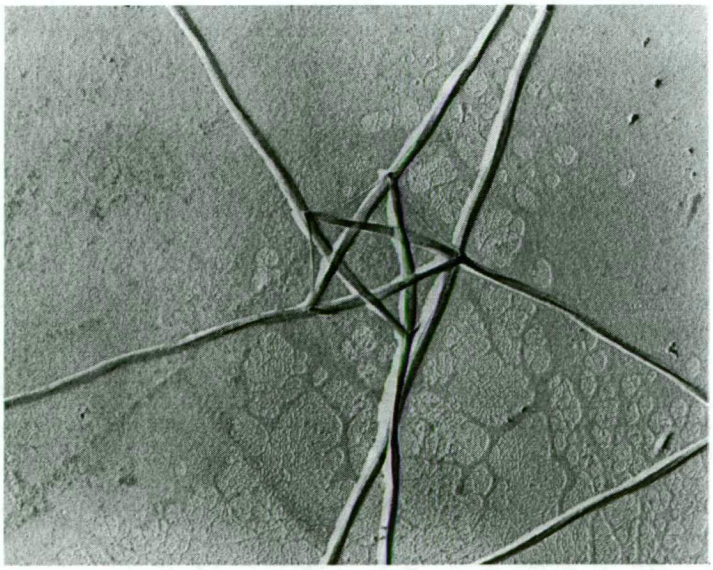


Fig. 4.117: *P. globosa* "pentagonal star" (c. 1.0 μm); from a Derwent enrichment culture

(Micrograph no: 4773)

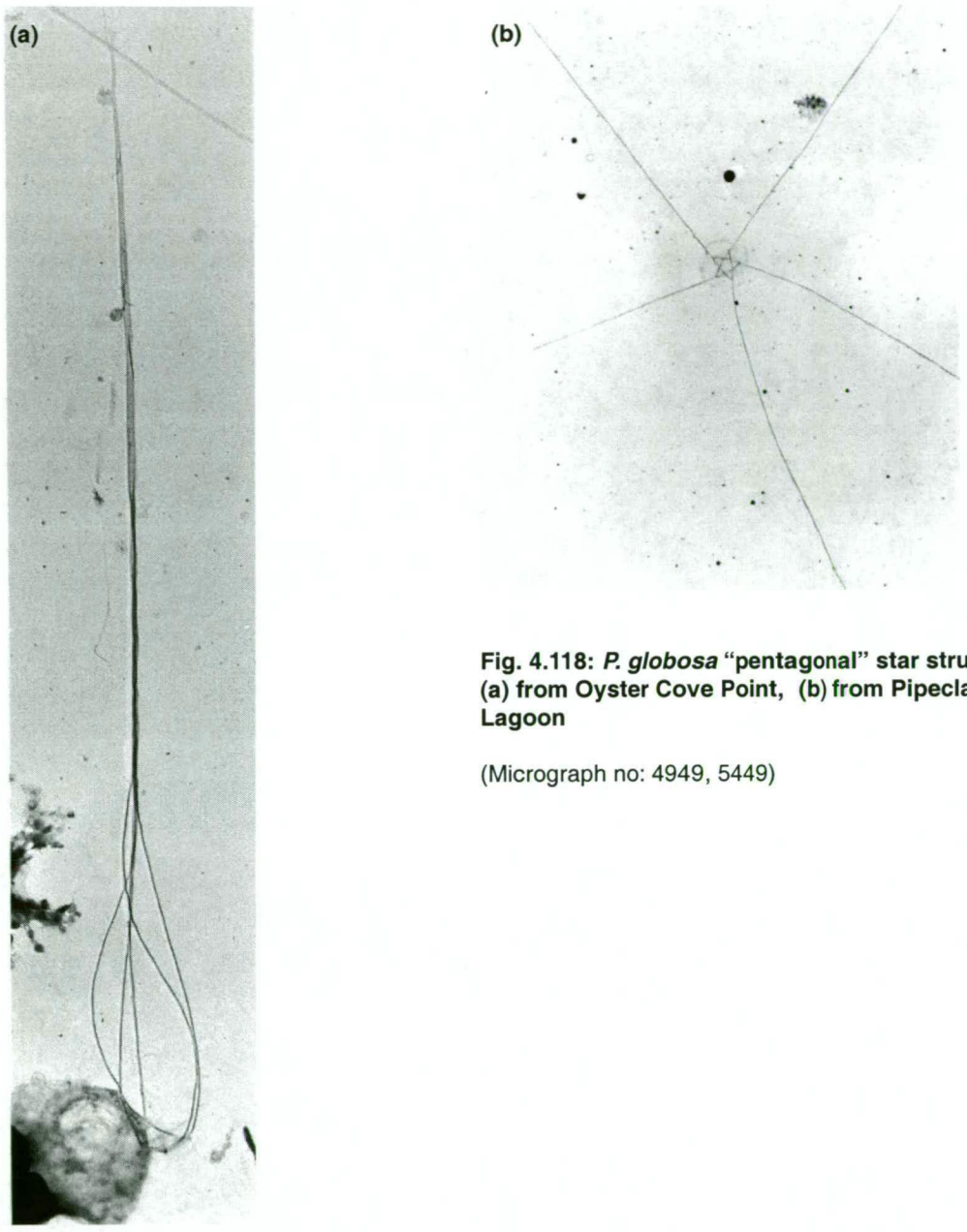


Fig. 4.118: ***P. globosa*** **"pentagonal" star structures; (a) from Oyster Cove Point, (b) from Pipeclay Lagoon**

(Micrograph no: 4949, 5449)

Fig. 4.119: ***P. globosa*** **small scales (c. 0.1 μm), and larger scales (0.2 x 0.18 μm); from a Derwent enrichment culture**

(Micrograph no: 4770)

***Phaeocystis scrobiculata* Moestrup** **Figs. 4.120 - 4.122**

Micrographs: Moestrup, 1979; Figs. 28 - 32.
Hallegraeff, 1983; Fig. 22.
Beech, 1983; Plate 2.15C.
Estep et al, 1984; Fig. 30.
Thomsen, 1986; Fig. 27.
Hoepffner and Haas, 1990; Fig. 3.

Present Findings.

Phaeocystis scrobiculata was found in samples from the Derwent River, Pipeclay Lagoon and Honey Moon Bay.

It was not found in any enrichment cultures, and has not yet been grown in laboratory culture (Vaulot et al, 1994).

Description.

The key feature used for identification of *P. scrobiculata* is its unique nine-rayed structure, consisting of four pairs of rays and one single ray (Moestrup, 1979). This structure, observed in Tasmanian material, was c. 2 μm in diameter, with the rays themselves being many times longer than the cell (Fig. 4.120). Each ray was less than 0.1 um thickness with a finely tapering tip.

Two types of scales were observed (Fig. 4.121): large oval scales with a peripheral upright rim, 0.6 - 0.7 x 0.4 - 0.6 μm ($\bar{x}$=0.6 x 0.5 μm; n=22); and smaller circular scales with a patternless rim, 0.25 - 0.3 μm in dimeter ($\bar{x}$=0.3 μm; n=22). These smaller scales were found between and under the larger scales (Fig. 4.122). Both scale types had a pattern of radiating ridges on the proximal side and a patternless distal side.

These observations agreed with previous descriptions made of this species (Table 4.33), although the small circular scales were slightly larger in the Tasmanian material than in the type species.

P. scrobiculata is known only as single cell and, unlike *Phaeocystis globosa*, no colony-forming stage has yet been observed.

Distribution.

P. scrobiculata is a widely distributed species and has been reported from tropical and temperate waters in both hemispheres, including: the North Atlantic Ocean, the North Pacific Ocean, and from Thailand, in the northern hemisphere (Estep et al, 1984; Thomsen, 1986; Hoepffner and Haas, 1990); New Zealand coastal waters, Victorian coastal waters and the East Australian Current, in the southern hemisphere (Moestrup, 1979; Beech, 1983; Hallegraeff, 1983)

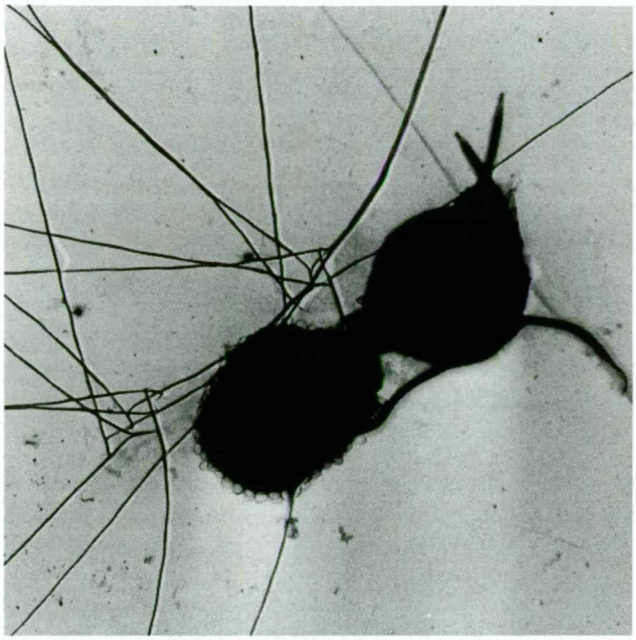


Fig. 4.120: *P. scrobiculata* cells and nine-rayed structures (c. 2 μm diam.); from Pipeclay Lagoon

(Micrograph no: 5015)

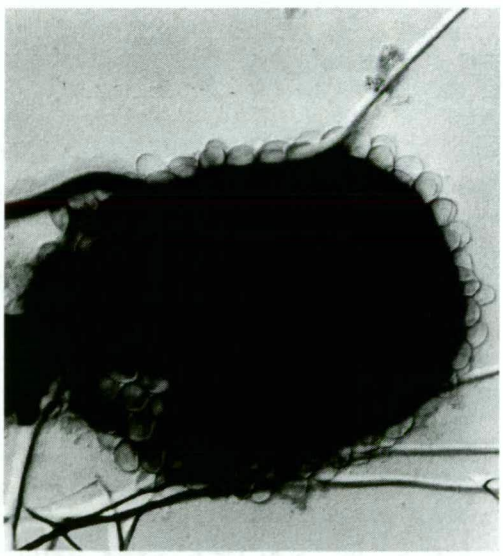


Fig. 4.121: *P. scrobiculata* scales on cell body; from Pipeclay Lagoon

(Micrograph no: 5015)

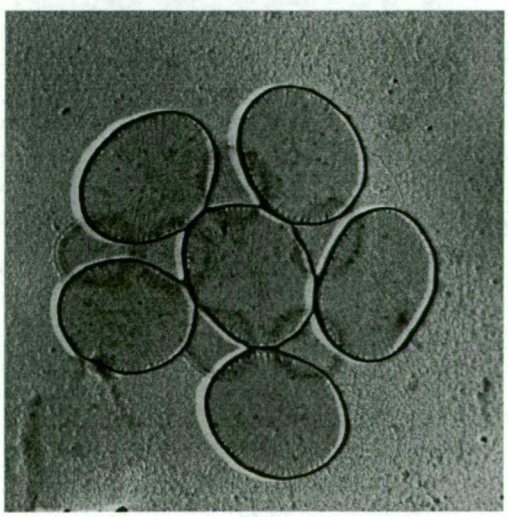


Fig. 4.122: *P. scrobiculata* small scales (c. 0.3 μm), and larger scales (0.6 x 0.5 μm); from the Derwent River

(Micrograph no: 5338)

Table 4.33: Comparison of the key features of *P. scrobiculata* from different locations.

SOURCE	NINE-RAY STRUCTURE	LARGE SCALES		SMALL SCALES
	Diam. (μm)	*Length (μm)*	*Width (μm)*	*Diam. (μm)*
New Zealand *(type)* (Moestrup, 1979)	2.0 (n=1)	0.6	0.45	0.19 - 0.21
East Australian Current (Hallegraeff, 1983)	c. 2.5 (n=1)	0.41	0.30	0.1
Victoria, Australia (Beech, 1983)	c. 2.5 (n=1)	-	-	-
North Atlantic Ocean (Estep et al, 1984)	2.1 (n=1)	0.4	0.3	-
Thailand (Thomsen, 1986)	1.7 (n=1)	-	-	-
North Pacific Ocean (Hoepffner & Haas, 1990)	c. 1.5 (n=1)	-	-	-
Tasmania	1.7 - 2.0 (n=2)	0.6 - 0.7 ($\bar{x}$ =0.6, n=22)	0.4 - 0.6 ($\bar{x}$ =0.5, n=22)	0.25 - 0.3 ($\bar{x}$ =0.3, n=22)

***Prymnesium* aff. *nemamethecum* Pienaar et Birkhead** **Fig. 4.123**

Micrographs: Pienaar and Birkhead, 1994; Figs. 25 - 27.

Present Findings.
Only one scale was observed from a Dru Point sample.

Description.
This scale resembled one of the three scale types of *Prymnesium nemamethecum.* It was elliptical, 0.74 x 0.38 μm in size, with a narrow marginal rim, and was patterned with radiating ridges arranged in quadrants (Fig. 4.123). There were c. 20 ridges per quadrant; these extended from a longitudinal central thickening to the scale edge.

This scale type is usually confined to the haptonema surface and is not as common as the other two scale types (Pienaar and Birkhead, 1994). To confirm species identification, it would be necessary to find other scales.

Distribution.
P. *nemamethecum* was described from full strength salinity water near Cape Town, South Africa (Pienaar and Birkhead, 1994) and has also been reported from Australia (Moestrup and Thomsen, 1995).

Toxicity.
The toxicity of this species is unknown. Of the seven well-characterised *Prymnesium* species, four are known to be toxic, and thus a bloom of any *Prymnesium* species should be considered harmful.

Fig. 4.123: *P.* aff. *nemamethecum* scale (0.7 x 0.4 μm); from Dru Point

(Micrograph no: 4825)

***Prymnesium patelliferum* Green, Hibberd et Pienaar** **Figs. 4.124 - 4.125**

Micrographs: Green et al, 1982; Figs. 18 - 21.
Beech, 1983; Plate 2.16 C - D.
Eikrem and Throndsen, 1993; Fig. 9.
Larsen et al, 1993; Figs. 2 - 6.

Location.
Scales and whole cells were found in a Little Swanport sample.

Prymnesium patelliferum grew in GSe enrichment cultures from Pipeclay Lagoon and three unialgal strains (CS-376/A, B, C) are currently maintained in the CSIRO Collection of Living Microalgae (at 15°C, under standard growth conditions).

Description.
Whole cells were round to oval, 4 - 5 x 3 - 4.5 µm in size. The two flagella were equal or subequal in length, ranging from 9 - 17 µm, and the short haptonema was 2 - 3 µm (Fig. 4.124).

Cells were covered with two similar scale types, with outer scales having an upright rim and inner scales, a narrow inflexed rim (Green et al, 1982). Scales were oval, and ranged from 0.29 - 0.42 x 0.25 - 0.32 µm ($\bar{x}$=0.35 x 0.3 µm; n=38) in culture, and from 0.41 - 0.45 x 0.25 - 0.29 µm ($\bar{x}$=0.4 x 0.3 µm; n=5) in the wild material. The upright rim was c. 0.05 - 0.06 µm high. Both scale types were patterned with radiating ridges arranged in quadrants, 11 - 14 ridges per quadrant, extending to the scale edge on the proximal surface and to the scale rim on the distal surface. A central longitudinal thickening was also found on the distal surface (Fig. 4.125).

There was more size variation in the cells found in this survey compared to the type material (Table 4.34), but in structural aspects, both these cells and type cells were the same. There were no major differences between cultured and wild cells. Cells grew easily and were more often seen in enrichment cultures than in wild samples, a finding which agreed with comments made by Beech (1983).

Based on similarities in scale morphology, as well as genetic studies, growth characteristics and toxicity, it has been suggested that *P. patelliferum* and *P. parvum* are actually different generations rather than separate species (Larsen and Edvardsen, 1998). Larsen (1999) proposed that *P. patelliferum* should be relegated to the status of a form of *P. parvum.*

Distribution.

P. patelliferum has been reported from temperate coastal waters in both hemispheres. It is known from the UK, Norway, Bulgaria, USA, Canada, China and Australia (Green et al, 1982; Beech, 1983; Chen and Zeng, 1986; Larsen et al, 1993; Eikrem and Throndsen, 1993, Guo et al, 1996).

Toxicity.

P. patelliferum is known to be toxic and has been implicated in harmful bloom events resulting in fish mortality (Green et al, 1982; Larsen et al, 1993; Edvardsen and Paasche, 1998).

Since 1989, annual blooms of *P. parvum* and *P. patelliferum* have occurred in the Sandsjord system on the southwest coast of Norway. Between 1989 and 1991, these blooms were responsible for the death of over 1250 tons of farmed salmon (Eikrem and Throndsen, 1993).

P. patelliferum is also toxic to *Artemia* nauplii (Larsen et al, 1993), and has sublethal effects on feeding and reproduction of the copepod, *Acartia clausi* (Nejstgaard and Solberg, 1996).

In the present work, *P. patelliferum* was found to be toxic to *Artemia* nauplii, as well as to hatchery-reared oyster larvae and local Derwent River decapod zoea (see Chapter 7).

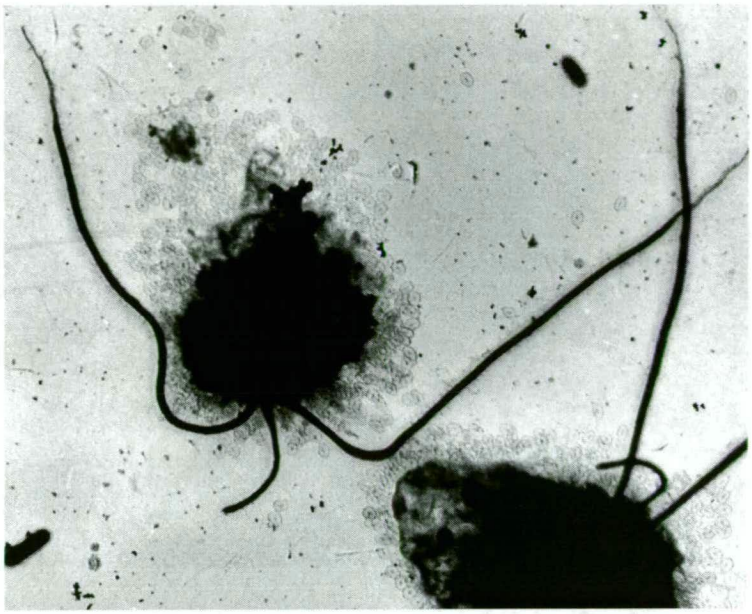


Fig. 4.124: *P. patelliferum* cells (c. 4 μm), with two flagella and haptonema; from a Pipeclay Lagoon enrichment culture

(Micrograph no: 4814)

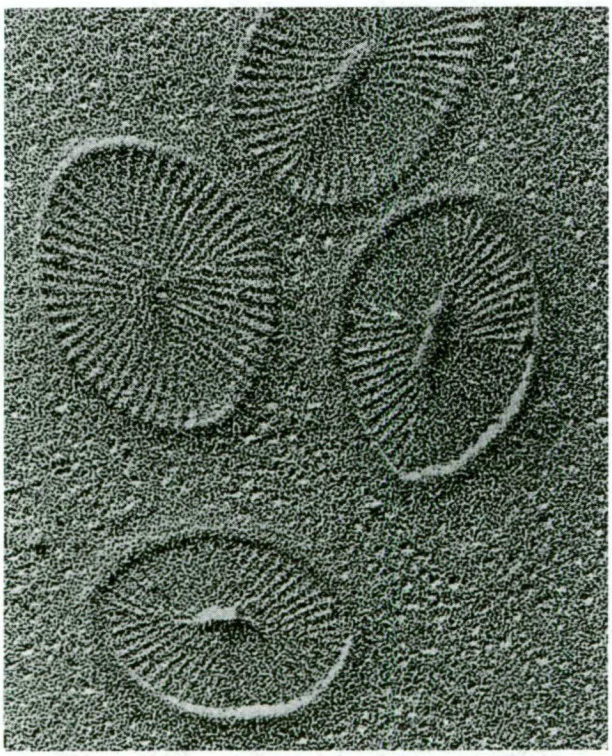


Fig. 4.125: *P. patelliferum* scales (c. 0.35 μm); from the same Pipeclay Lagoon enrichment culture

(Micrograph no: 4813)

Table 4.34: Scale sizes of *Prymnesium patelliferum* from different locations.

SOURCE	SCALE DIMENSIONS	
	Length (µm)	*Width (µm)*
UK *(type)* (Green, 1982)	0.36 - 0.37	0.25 - 0.27
Norway (Larsen et al, 1993)	0.38 - 0.40 ($\bar{x}$ =038; n=9)	0.23 - 0.29 ($\bar{x}$ =0.26; n=9)
Norway (Eikrem & Throndsen, 1993)	0.38 - 0.40 ($\bar{x}$ =0.39; n=3)	0.25 - 0.28 ($\bar{x}$ =0.26; n=3)
Victoria, Australia (Beech, 1983)	0.38 - 0.44 ($\bar{x}$ =0.41; n=6)	0.26 - 0.29 ($\bar{x}$ =0.28; n=6)
Tasmania, Australia	0.29 - 0.42 ($\bar{x}$ =0.38; n=43)	0.22 - 0.32 ($\bar{x}$ =0.27; n=43)

Pavlova sp. **Figs. 4.126 - 4.127**

Present Findings.
Cells were found in ML enrichment cultures derived from Pipeclay Lagoon, Southport and Coles Bay samples.

Unialgal isolates were established in culture using a combination of micropipetting and serial dilution. Three strains of *Pavlova* (CS-491, CS-492/8 and CS-492/3) are currently maintained in the CSIRO Collection of Living Microalgae (in GSe medium at 18°C, under standard growth conditions).

Description.
Cells were identified as belonging to the genus *Pavlova* based on the presence of the following features: a long flagella, c. 10 μm, with tiny knob scales and fine hairs; a short flagella, c. 3.5 μm; and a reduced haptonema, less than 1 μm in length (Figs. 4.126, 4.127). Cells themselves were 3 - 5 μm.

Of the ten described species of *Pavlova*, only one (*P. pinguis*) is predominantly from the marine environment, with the remaining species from brackish water or freshwater habitats (Green, 1980). Thus, it was likely that the *Pavlova* species observed in this study was *Pavlova pinguis*, but to confirm this, ultrastructural studies are required.

Distribution.
Pavlova is a widespread, but infrequently recorded, species found in various habitats from freshwater lakes to marine environments, although most species thrive in brackish waters (Green, 1980).

Pavlova species have been reported from the Baltic Sea (Edler et al, 1984; Jochem, 1990), coastal waters of Norway and the UK (Throndsen, 1969; Parke and Dixon, 1976), Indonesian mangrove forests (Ogino and Chihara, 1988), the East Australian Current (Hallegraeff, 1983), and have been isolated from the Gulf Stream, the Sargasso Sea, the North Atlantic Ocean and the Indian Ocean (Andersen et al, 1991)

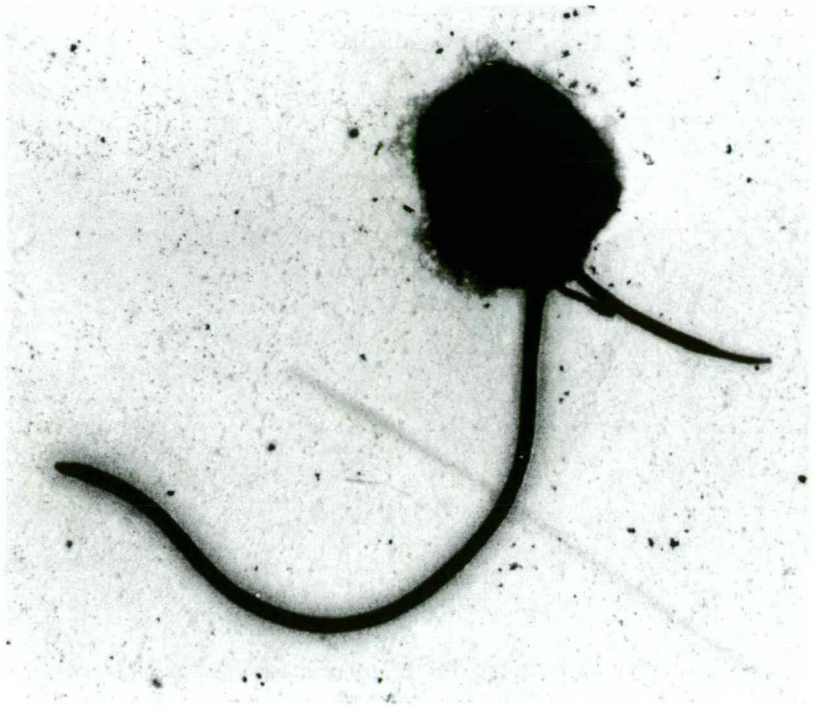


Fig. 4.126: *Pavlova* sp., cell (c. 5 μm), with unequal flagella and reduced haptonema; from Pipeclay Lagoon

(Micrograph no: 5045)

Fig. 4.127: *Pavlova* sp., flagellum showing "knob" and hair scales; from Pipeclay Lagoon

(Micrograph no: 5045)

***Pavlova pinguis* Green** **Figs. 4.128 - 4.133**

Micrographs: Green, 1980; Figs. 1 - 16, 43 - 46.

Present Findings.
Cells were seen in GSe enrichment cultures derived from Pipeclay Lagoon.

Unialgal isolates were established in culture using a combination of micropipetting and serial dilution. Three strains of *Pavlova pinguis* (CS-375/1, CS-375/3 and CS-375/5) are currently maintained in the CSIRO Collection of Living Microalgae (in GSe medium at 18°C, under standard growth conditions).

This is a new record for Australian waters.

Description.
Cells were approximately 5 x 3 μm in size, and had two unequal flagella. The long flagellum, 10 -13 μm ($\bar{x}$=11.5 μm; n=3) had fine hairs, c. 1.2 μm, knob scales, c. 0.04 μm, and a tapered tip (Fig. 2.129). Unlike the long flagellum, the short flagellum, 3 - 4 μm ($\bar{x}$=3.7 μm; n=3), did not have hairs or knob scales, but did have a tapered tip. The haptonema was short and straight, with a length of 1.25 - 1.3 μm ($\bar{x}$= 1.28 μm; n=3).

Under the light microscope, cells were seen to swim with the characteristic spinning of *Pavlova* cells. The long flagellum had a undulating wave-like action, while the short flagellum had a stiff jerking motion.

To differentiate to species level, it was necessary to examine cell ultrastructure. In longitudinal section, a bulging pyrenoid with a cytoplasmic intrusion was observed at the posterior end of the cell (Fig. 4.128). On the inner face of the chloroplast, and near the flagellar bases, there was a stigma (or eyespot), composed of at least ten large globules arranged in a concave configuration (Fig. 4.130). The Golgi apparatus had a prominent position at the centre of the cell, near the nucleus which had a triangular profile in longitudinal section (Fig. 4.128). Lamellar vesicles and two storage bodies were found at the posterior of the cell, while at the anterior of the cell, the flagella and haptonema were seen. The hatonema had a slight bulge at its base, the "posterior swelling", and lacked hairs or scales. Small round knob scales were apparent on the long flagellum, but not on the short flagellum (Fig. 4.132).

In transverse section, the large bi-lobed chloroplast was seen to be joined at the base by the pyrenoid (Fig. 4.131), and the microtubular arrangement of the long flagellum (nine pairs of outer microtubules and two individual inner ones) was observed (Fig. 4.133).

The presence of both a pyrenoid and a stigma indicated that this species was *P. granifera*, *P. gyrans* or *P. pinguis*, as other *Pavlova* species have either a pyrenoid, or a stigma, or lack both (Green, 1980). *P. granifera* was an unlikely candidate as it is a freshwater species, with a dominant non-motile life stage. Motile cells, when observed, have small body scales, and are larger than the Tasmanian material, with longer flagella and haptonema. *P. gyrans* is reported from brackish water habitats and has motile cells which are elongate and variable in shape, in constrast to the consistently oval Tasmanian cells. Consequently, it was most likely that the Tasmanian species was *P. pinguis,* given the similarities in size, shape, flagellar length, and internal structure.

There were some minor differences between the Tasmanian material and the type description of *P. pinguis* (Green, 1980). Although the long flagellum had knob scales, they were not "dumb-bell shaped" as described for the type species, nor was the slender terminal filamant on the haptonema observed.

Distribution.

Pavlova pinguis is difficult to identify without examining live cells or studying ultrastructure. The cell illustrated by Hallegraeff (1983; Fig. 23) from the East Australian Current could well be *P. pinguis*, given its size and the presence of a terminal filament on the haptonema.

Cultures of *P. pinguis* have been established from the North Atlantic Ocean (Green, 1980; Andersen et al, 1991), with samples collected at approximately the same latitude, 35°N, but at different longitudes, 16 and 65°W, from both sides of the North Atlantic Ocean. The cultures established in this study were from samples collected at a similar but southerly latitude, 42°S, and it is possible that this species is confined to temperate waters.

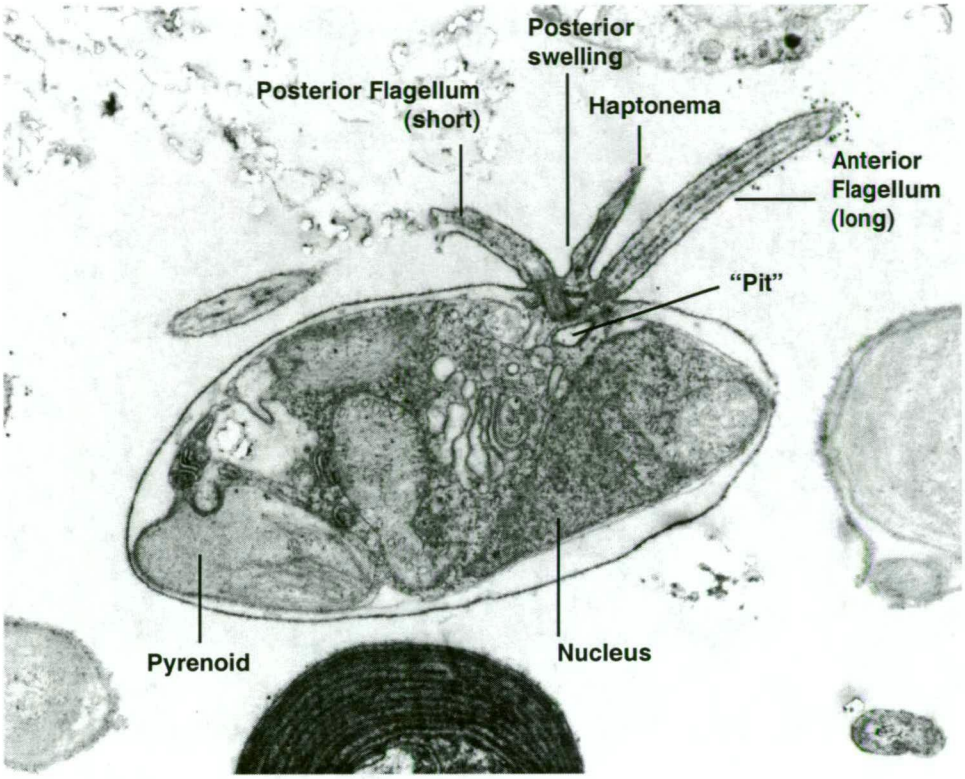


Fig. 4.128: Longitudinal section of *P. pinguis* (c. 5 μm)

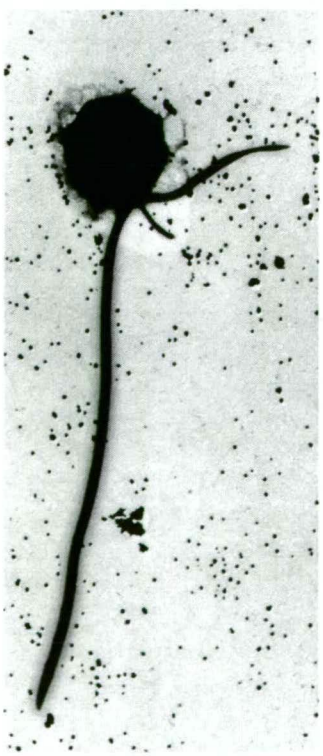


Fig. 4.129: *P. pinguis* whole cell (c. 3 μm), with long and short flagella, and haptonema; from Pipeclay Lagoon

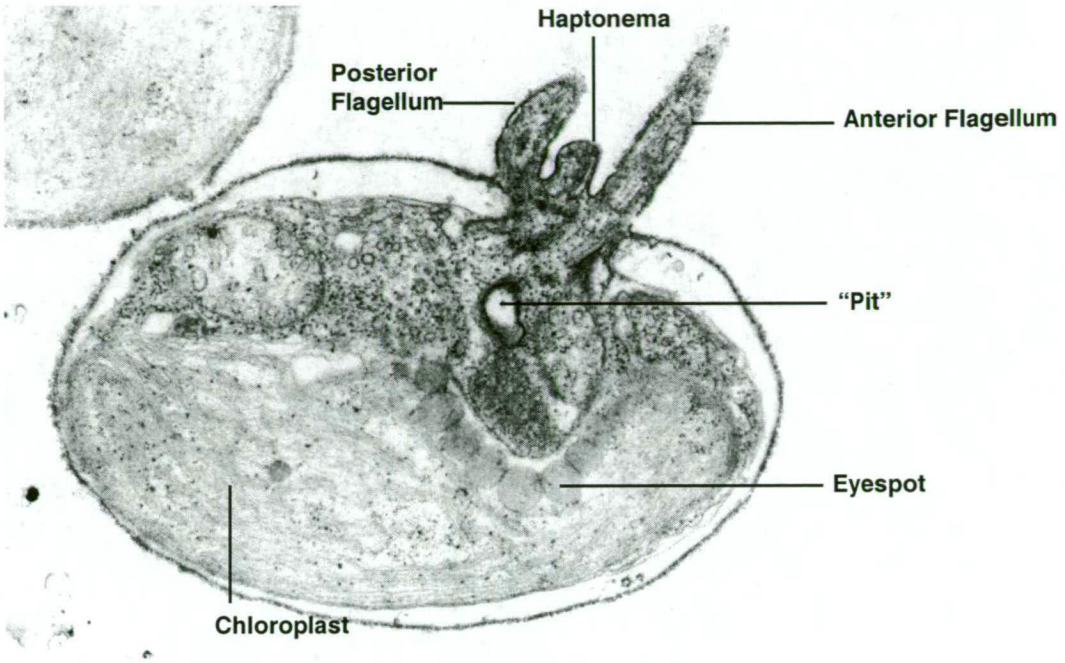


Fig. 4.130: Longitudinal section of *P. pinguis*

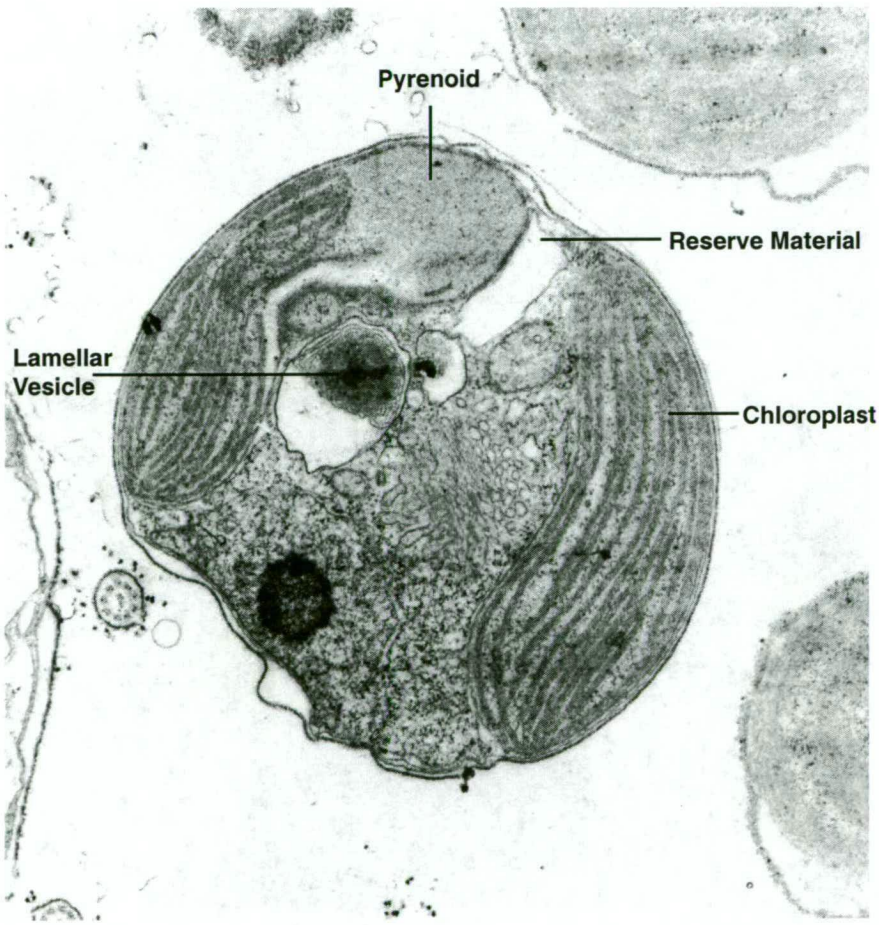


Fig. 4.131: Transverse section of *P. pinguis*

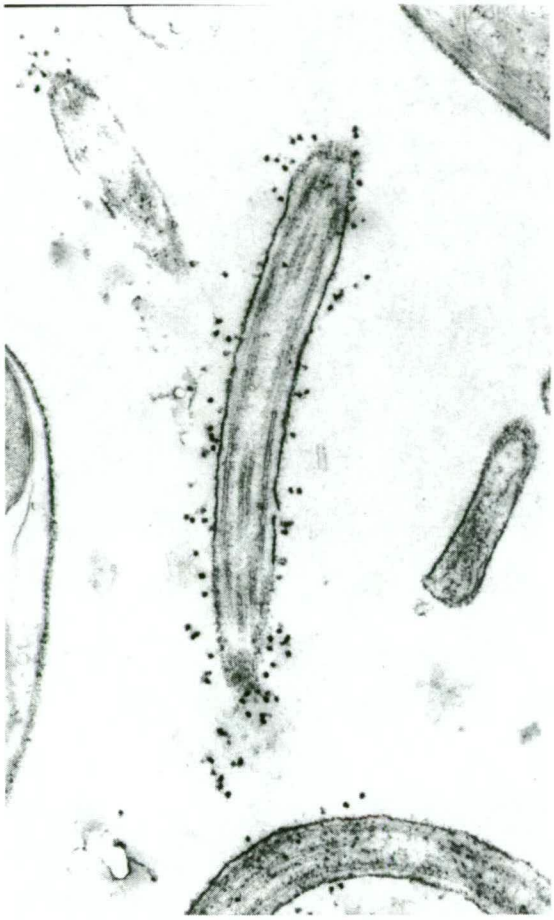


Fig. 4.132: Longitudinal section of *P. pinguis* flagellum showing "knob" scales

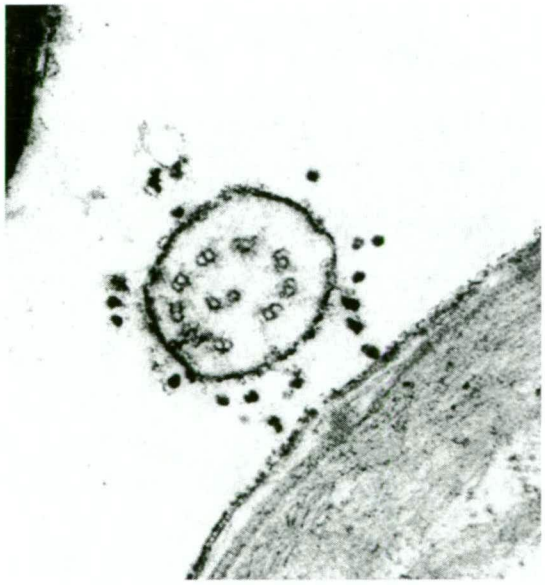


Fig. 4.133: Transverse section of *P. pinguis* flagellum showing microtubules and "knob" scales

4.4 Discussion

In this survey, 37 prymnesiophyte species well characterised in the literature were found, in addition to over 20 types of previously unillustrated scales. This showed the biodiversity of this class in southern Tasmanian waters.

Of the 55 *Chrysochromulina* species described in the literature, 30 were found in this survey, reflecting findings in the northern hemisphere, where 38 species have been found in Scandinavian waters alone, with an additional 30 forms reported, but not yet described, in the literature (Eikrem and Edvardsen, 1999). It is estimated that there could well be over 100 species of *Chrysochromulina* world-wide.

Many of the prymnesiophytes seen in this survey have previously been reported from Australian and New Zealand coastal waters (Beech, 1983; Hallegraeff, 1983; Rhodes et al, 1996), but there were many different scale types seen which have not been illustrated in the literature. Unfortunately, much of this new material could not be fully characterised, as whole cells complete with scales and flagella, were seldom found. This may have been due to the sampling preparation techniques used. Continous plankton centrifuging is possibly too vigourous a method to use for fragile cells, and gravity filtration has been recommended as a gentler alternative (J. Throndsen. pers. comm.).

Many species found in this survey have also been reported from the northern hemisphere, with *Chrysochromulina novae-zelandiae* being the only species to be found solely in southern waters of Australia and New Zealand. New records from the present study for the southern hemisphere were *C.* aff *scutellum*, *C.* aff *brachcylindra* and *C.* aff. *ahrengotii*.

In additional to new scale types illustrated in this study, many scales had morphological similarities to described species, but retained enough differences to warrant uncertainity in attributing the scales to that particular species. Examples include scales described as having affinities to *C. acantha*, *C. ahrengotii*, *C. brachycylindra*, *C. chiton*, *C. polylepis*, C. *scutellum*, *C. simplex* and *C. vexillifera.*

Additional information on *C. adriatica* and *C. mactra* scales was obtained from the present work, with distal patterning being seen for the first time on *C. adriatica* scales, and scale sizes reported for the small scales of *C. mactra* (1.2 x 0.8 μm).

Generally, scale structure was similar to type material, but there were some exceptions. Spine scales of *C. hirta* from Australian waters were smaller (9 - 17 μm) in comparison to the type scales from northern waters. Similarly, *C. parkae* had shorter spines and wider rims in Australian material. Eikrem and Moestrup (1998) listed differences in scale morphology for *C. brevifilum* from the UK and South Africa, and for *C. eppihipium* from the UK and Denmark. It is possible that different biogeographical forms of certain species exist.

At least half the species observed in the present study grew in enrichment culture, but only a small number of strains were able to be maintained in culture long term. This is reflected in the fact that there is only a small percentage of prymnesiophyte species deposited in culture collections world-wide, in comparison to the number of species described from the wild.

It is likely that different species have specific growth requirements which are not being adequately met under standard culturing conditions. *Phaeocystis scrobiculata* has not been cultured whereas *P. globosa* grows readily in enrichment culture. *Corymbellus aureus* was described initially by Green (1976) from cultures grown in Erdschreiber medium, but all other reports have been from wild material, suggesting that this species may have particular nutrient requirements not usually supplied in typical algal culture medium. Erdschreiber medium contains soil extract which can vary substantially in composition depending on the locality from which it is obtained.

As some *Chrysochromulina* species are known to be mixotrophic, using higher concentrations of organic substances to promote bacterial growth, or alternatively providing a prey species, may be useful. It was interesting to note that in this study, *Chrysochromulina* cultures in petri dishes survived for longer periods of time than their counterparts in glass flasks or test-tubes. Cultures in flasks usually needed to be subcultured every two weeks, whereas cells in petri dishes were still viable up to six months after initial subculture.

In contrast, *Prymnesium patelliferum* was found to be more common in enrichment cultures than in wild samples. The growth of this species is promoted by eutrophic conditions (Edvardsen and Paasche 1998). The two sites where *P. patelliferum* was found in this survey were both heavily involved in oyster farming and both had nursery facilities for juvenile production. Further samples would be required to determine if this was just coincidence or if growth conditions were influenced by oyster farming activities.

In culture, there generally appeared to be less scale variability in comparison with wild material. For example *C. parkae* and *C. pringsheimii* had a range of spine lengths in wild samples, but smaller and consistent lengths in culture. It would be interesting to examine changes in scale morphology with time in culture, and to see if there are differences in morphology as well as size. Eikrem and Throndsen (1998) reported consistent scale morpholgy in *C. leadbeateri* cultures compared with field material over 6 - 7 years.

Scale morphology is still the most useful criteria for identifying prymnesiophytes, with genetic studies confirming taxa based on morphological structure and ultrastructure (Medlin et al, 1994, Edvardsen et al, 2000). However, the range of variability in scale morphology is often confusing, leading to species misidentification. One possibility is that this variation may be the result of different life cycle stages, and further investigation is required to find if distinguishable morphotypes can be closley linked, as has been proposed for *Prymnesium parvum* and *P. patelliferum* (Larsen, 1999).

5.0 DIVISION CHLOROPHYTA: CLASS PRASINOPHYCEAE

Prasinophytes are small green flagellates characteristically covered with numerous organic scales. They are found mainly in marine environments, both coastal and oceanic, but there are also some freshwater species.

5.1 Taxonomic Features

Useful taxonomic features of prasinophytes include cell symmetry, the presence of scales or theca, the number of layers of body and flagellar scales, the number and insertion of flagella, and possession of a non-motile phycoma stage.

Cells are round to oval, quadrangular to pyramidal in shape and are sometimes flattened or bilaterally compressed. Flagellated cells possess one, two, four or eight (occasionally 16) flagella, which usually emerge from a depression, the "flagellar pit", at the anterior of the cell. During swimming, flagella may act in the same way (homodynamic), or each may move differently (heterodynamic), to pull the cell. Flagella may beat with an undulating action, as in *Pseudoscourfieldia*, while the flagellar motion of *Pyramimonas* and *Tetraselmis* is similar to "breast stroke" swimming, showing effective and recovery strokes for forward movement (Inouye and Hori, 1991).

Scales are found on the flagella, giving them a rather stiff and thick appearance, and on the cell body. These scales have several different morphologies and are arranged in one or more layers. Details are best seen in stained or sectioned material under the transmission electron microscope.

The genera, *Dolichomastix*, *Mamiella* and *Mantoniella*, have relatively simple scale structures, usually consisting of one layer of comparable "spider-web" scales on the cell body and flagella (Manton, 1977; Moestrup, 1984; Marchant et al, 1989). *Pseudoscourfieldia* has two layers of scales covering the cell and flagella: an inner layer of small square or pentagonal scales; and an outer layer of rod-shaped scales in double rows (Moestrup and Throndsen, 1988).

In contrast, *Pyramimonas* has a complex scale structure, with up to seven different scale types arranged in three layers on the cell body and two layers on the flagella. The cell body is typically covered with by three layers of scales: an innermost layer of small square scales; an intermediate layer of box scales; and an outermost layer of

crown or "basket-like" scales. Additional scale layers may be present, for example, the "footprint-scales" observed by McFadden et al (1982) for the Antarctic species, *Pyramimonas gelidicola.* The flagella are covered by three different types of scales: an innermost layer of small pentagonal scales arranged in helical rows; external layers of overlapping longitudinal rows of larger mesh-work, or limuloid, scales; and long tubular hair scales, usually in two opposite rows.

Stellate scales are found in the genus *Nephroselmis,* while in *Tetraselmis*, stellate-like scales are fused together to form a theca (Moestrup and Ettl, 1979; Norris et al, 1980; Inouye and Pienaar, 1984).

Flagellar hair scales are universally found within the Prasinophyceae, and have a high degree of ultrastructural complexity that appears to be conserved within genera (Marin and Melkonian, 1994). A single flagellum may have more than one type of hair scale.

One or (two) lobed chloroplasts are found in flagellated prasinophytes, with non-motile phycoma stages having numerous disc-shaped chloroplasts. Thylakoids occur in bands of two to six and interconnecting thylakoids are common. Most prasinophytes have a single eyespot, located at the periphery of the chloroplast. In several smaller species of *Pyramimonas*, the occurrence of two eyespots lying side by side, but on different lobes of the chloroplast, is common (McFadden et al, 1986).

Trichocysts, or ejectosomes, have been observed in some prasinophyte genera, including *Mamiella*, *Mantoniella* and *Pyramimonas* (Moestrup, 1984; McFadden et al, 1986; Marchant et al, 1989). They are roughly spheroidal, membrane-bound structures usually located at the periphery of the cell. Such organelles are atypical of the green algae and their function is uncertain.

Resting cysts are formed by various prasinophytes, the most common form being the large phycoma stage dominant in the life cycles of *Halosphaera*, *Pachysphaera* and *Pterosperma.* The phycoma grows to a size many times that of the original flagellated cell; this growth is accompanied by cell division within the phycoma, from which numerous motile cells are eventually released.

Marine representatives of scale-bearing nanoflagellates are found in several prasinophyte genera as outlined in Table 5.1.

Table 5.1: Classification of nanoflagellate prasinophytes from the marine environment (Throndsen, 1997).

CLASS: PRASINOPHYCEAE Moestrup et Throndsen 1988

Order: Mamiellales Moestrup 1984
- contains species lacking typical inner scales

Family: Mamiellaceae Moestrup 1984
- usually with "spider-web" scales

Genus: *Dolichomastix* Manton 1977
- with one type of body scale (4 spp)

Genus: *Mamiella* Moestrup 1984
- with two types of body scales, and two slightly subequal heterodynamic flagella (1 species)

Genus: *Mantoniella* Desikachary 1972
- with two types of body scales and very unequal flagella (2 spp)

Order: Chlorodendrales Fritsch 1917
- flagella and cell body covered by an underlayer of square or diamond-shaped scales

Family: Chlorodendracaea Oltmanns 1904
- flagellar underlayer scales covered by small (usually) rod-shaped scales

Genus: *Nephroselmis* Stein 1878
- flattened cells with two heterodynamic flagella (c. 6 spp)

Genus: *Pseuodscourfieldia* Manton 1975
- two homodynamic flagella (1 species)

Genus: *Tetraselmis* Stein 1887
- cells with scale "theca" and four flagella (c. 10 spp)

Family: Halosphaeraceae Haeckel 1894
- underlayer scales on the flagella are covered by meshwork scales in 9 longitudinal rows

Genus: *Cymbomonas* Schiller 1913
- bilateral symmetry and 4 long flagella (3 spp)

Genus: *Pachysphaera* Ostenfeld 1899
- best known from the phycoma or non-motile stage

Genus: *Pterosperma* Pouchet 1893
- best known from the phycoma or non-motile stage

Genus: *Halosphaera* Schmitz 1878
- best known from the phycoma or non-motile stage

Genus: *Pyramimonas* Schmarda 1850 emend. McFadden 1986
- quadilateral symmetry and pyramidoidal cells with four flagella (c. 50 spp, most from marine plankton)

5.2 Australian Findings

Prasinophytes from Australian coastal waters have been studied by Hallegraeff (1983), McFadden et al (1986, 1987) and Moestrup and Hill (1991, 1993), who identified *Mantoniella squamata*, *Nephroselmis minuta*, *N. rotunda* and 16 *Pyramimonas* species, including descriptions of six new species (Table 5.2). Blooms of *Pyramimonas disomata* and *P. cirolanae* were reported in brackish water from Paterson Lakes, Victoria (McFadden et al, 1986), and *Pyramimonas* blooms have also occurred in the Derwent River, Tasmania.

In this survey, 10 prasinophyte species were identified, of which three were new records for Australian waters (Table 5.3). Three potentially new *Pyramimonas* species were illustrated and five new types of box scales were recorded.

Table 5.2: Nanoflagellate prasinophyte species previously reported from Australian waters.

Species	Location	Reference
Mantoniella squamata (Manton et Parke) Desikachary	East Australian Current (coastal and oceanic)	Hallegraeff, 1983
Nephroselmis minuta (Carter) Butcher	Merimbula, NSW	Moestrup (pers. comm.)
Nephroselmis rotunda (Carter) Fott	Merimbula, NSW	Moestrup (pers. comm.)
Pyramimonas		
P. amylifera Conrad	Victorian coastal waters (8 sites)	McFadden et al, 1986
P. cirolanae Pennick	Victorian coastal waters (6 sites)	McFadden et al, 1986
P. cordata* McFadden	East Australian Current, Victorian coastal waters, (15 sites), common in Port Phillip Bay during spring	Hallegraeff, 1983 McFadden et al, 1986
P. disomata Butcher	Victorian coastal waters (14 sites), common in brackish waters	McFadden et al, 1986
P. grossii (Parke) Manton	East Australian Current, Victorian coastal waters (18 sites)	Hallegraeff, 1983 McFadden et al, 1986
P. longicauda (Van Meel) Inouye et Chihara	Victorian coastal waters (4 sites)	McFadden et al, 1986
P. mantoniae* Moestrup et Hill	Corner Inlet, Victoria	Moestrup and Hill, 1993
P. mitra* Moestrup et Hill	Corner Inlet, Wilson's Promentary, Victoria	Moestrup and Hill, 1991
P. moestrupii* McFadden	Victorian coastal waters, (10 sites), common in Hobson's Bay	McFadden et al, 1986
P. nephroidea* McFadden	Victorian coastal waters (4 sites)	McFadden et al, 1986
P. obovata Carter	East Australian Current	Hallegraeff, 1983
P. oltmannsii Schiller	Corner Inlet, Victoria	Moestrup (pers. comm.)
P. orientalis Butcher	Victorian coastal waters (4 sites)	McFadden et al, 1986
P. parkae Norris et Pearson	Victorian coastal waters (3 sites)	McFadden et al, 1986
P. propulsa* Moestrup et Hill	Sea Spray, Victoria	Moestrup and Hill, 1991
P. virginica Pennick	Victorian coastal waters (2 sites)	McFadden et al, 1986

* New species

Table 5.3: Nanoflagellate prasinophyte species found in southern Tasmanian waters and their overall distribution.

Species	Present Findings	Growth in Enrichment Media	New Record for Australia	Distribution
Dolichomastix nummulifera Manton	PCL, EN	-	Yes	Polar to tropical, coastal
Dolichomastix* aff. *tenuilepis Throndsen et Zingone	EN	-	Yes	Temperate, coastal (one report only)
Mamiella gilva (Parke et Rayns) Moestrup	DER, DP, PCL, HMB	GSe, GSe/2	Yes	Temperate to tropical, coastal
Mantoniella squamata (Manton et Parke) Desikachary	OCP, FP, DB, RB, PCL	-	-	Polar to tropical, coastal and oceanic
Pyramimonas amylifera Conrad	SPT, HMB	-	-	Temperate, coastal (common)
Pyramimonas cirolanae Pennick	DER, PCL	GSe	-	Subpolar to temperate, coastal (brackish water blooms)
Pyramimonas grossii (Parke) Manton	DER, STB, DP, OCP, FP, DB, SPT, PCL, RB, EN, HMB, LSP	GSe, GSe/2, GSe/10, ML	-	Temperate, coastal (common)
Pyramimonas longicauda (Van Meel) Inouye et Chihara	DER, PCL, OCP	-	-	Temperate, coastal (3 records)
Pyramimonas obovata Carter	PCL, DP, HMB, STB	-	-	Temperate, coastal and oceanic
Pyramimonas virginica Pennick	OCP, FP, DB, RB	-	-	Subpolar to tropical, coastal
***Pyramimonas* spp.** (8)	DER, PCL, RB, FP, SP, SPT, CB, HMB, EN		Yes	

5.3 Species Descriptions

***Dolichomastix nummulifera* Manton** **Fig. 5.1**

Micrographs: Manton, 1977. Figs. 1- 8.
Thomsen, 1986. Figs. 39 - 40.
Throndsen and Zingone, 1997. Figs. 38 - 40.

Present Findings.
Scales were found in Pipeclay Lagoon and Eaglehawk Neck samples.

This is a new record for Australian waters.

Description.
Dolichomastix nummulifera has very delicate body and flagellar scales, both with a pattern of concentric rings. The scales observed matched the type description of flagellar scales (Manton, 1977), being oval, 0.3 x 0.2 μm (n=2), each with five concentric rings and a slightly thickened margin (Fig. 5.1).

Distribution.
This species has been previously recorded from coastal waters around Denmark, Alaska, Arctic Canada, West Greenland, South Africa, Thailand and the Mediterranean Sea (Throndsen and Zingone, 1997, and references therein; Smith and Hobson, 1994).

D. nummulifera appears to be very robust species. It has been reported to have a wide temperature tolerance, ranging from -1°C at Resolute Bay, Canada, to over 20°C in the Mediterranean Sea (Manton, 1977; Throndsen and Zingone, 1997). It has been collected from surface waters as well as at depths of up to 20 m, suggesting it can also tolerate a wide range of light intensities.

However, *D. nummulifera* has been rarely recorded and then usually only as preserved material. It has not yet been cultured in the laboratory and further information on living cells under the light microscope is required.

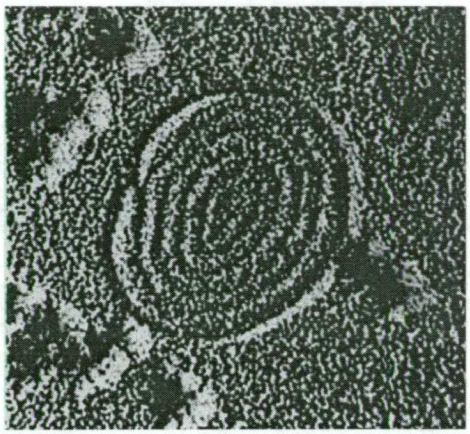


Fig. 5.1: *D. nummulifera* scale (0.3 x 0.2 μm); from Pipeclay Lagoon

(Micrograph no: 4853)

***Dolichomastix* aff. *tenuilepis* Throndsen et Zingone** **Figs. 5.2 - 5.3**

Micrographs: Throndsen and Zingone, 1997. Figs. 25 - 37.

Present Findings.
One cell with body and flagellar scales was observed in the Eaglehawk Neck sample.

This is a new record for Australian waters.

Description.
Cell size was c. 3 µm and flagellar length was 10 µm (Fig. 5.2a). Only one flagellum was seen on the cell, but it was possible that the other flagellum became detached during sample preparation.

Three types of scales were seen. On the cell body, round scales, 0.3 µm (n=3), had a surface pattern of at least 10 concentric rings surrounding a central hole, and a thickened margin (Fig. 5.3). On the flagella, scales were irregularly elliptical, 0.3 x 0.2 µm (n=4), with at least 5 concentric rings and a thickened margin. Short tubular hair scales were 0.4 - 0.5 µm in length ($\bar{x}$=0.43 µm; n=4), with thread-like distal tips ranging from 0.11 - 0.18 µm ($\bar{x}$=0.15 µm; n=3) (Fig. 5.2).

This cell was similar to that described for *D. tenuilepis* (Table 5.4). However, there were a number of differences. Two sizes of body scales, 0.4 and 0.3 µm, are present on cells of *D. tenuilepis*, but only the larger size was seen on the Tasmanian specimen. On the flagella, the short tubular hairs of *D. tenuilepis* had longer distal tips in comparison to the Tasmanian material, and basal flagellar hairs were not seen.

Distribution.
D. tenuilepis was originally described from the Mediterranean Sea, off the Island of Capri (40° 30' N and 40°49' N), at water temperatures ranging from 15 - 25°C and an average salinity of 37.8 psu (Throndsen and Zingone, 1997). The cell described here was found at a similar southern latitude.

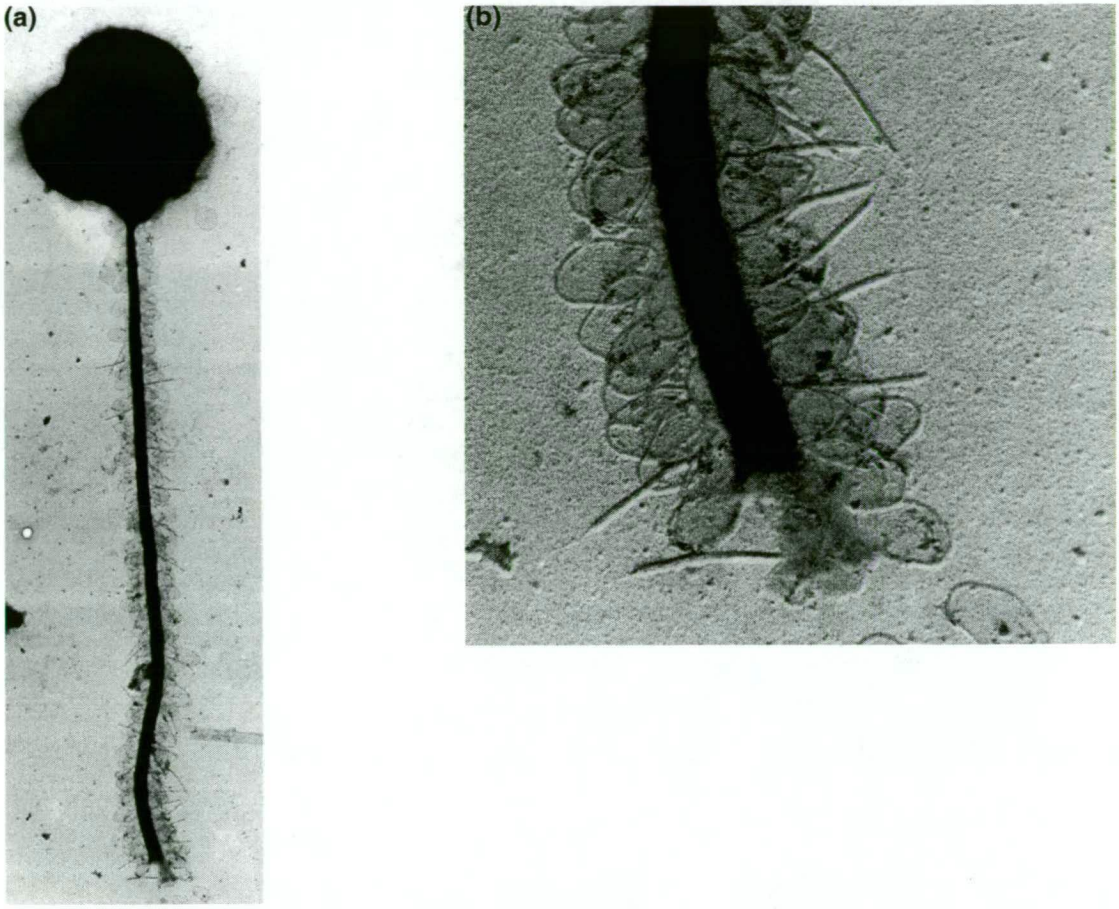


Fig. 5.2: (a) *D. aff. tenuilepis* cell (c. 3 μm; flagellum 10 μm) from Eaglehawk Neck; (b) flagellar detail showing scale types

(Micrograph no: 5420 and 5422)

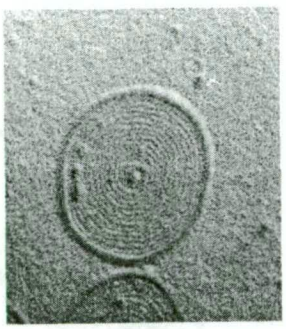


Fig. 5.3: *D. aff. tenuilepis* body scale (0.3 μm)

(Micrograph no: 5421)

Table 5.4: Scales of *Dolichomastix tenuilepis* compared with those of *Dolichomastix* aff. *tenuilepis*.

SOURCE	CELL DIMENSIONS	FLAGELLAR LENGTHS	BODY SCALES *(No. of Concentric Rings)*	FLAGELLAR SCALES *(No. of Concentric Rings)*	FLAGELLAR HAIR SCALES
Dolichomastix tenuilepis	3 - 4.5 μm	**(1)** 12 - 18 μm	**(1)** 0.4 μm	0.3 x 0.2 μm	0.4 μm; tips 0.3 μm
Mediterranean Sea		**(2)** 7.5 - 16 μm	(c. 14)	(7 - 10)	Basal hair scales, 1.7 μm.
(Throndsen & Zingone, 1977)			**(2)** 0.3 μm		
			(c. 9)		
Dolichomastix* aff. *tenuilepis	c. 3 μm	10 μm	0.4 μm	0.3 x 0.2 μm	0.4 μm; tips 0.11 - 0.18 μm
Tasmania, Australia	(n=1)	(n=1)	(n=3)	(n=4)	($\bar{x}$ =0.43 & 0.15 μm; n=4)
			(≥ 10)	(≥ 5)	No basal hair scales seen.

***Mamiella gilva* (Parke et Rayns) Moestrup** **Figs. 5.4 - 5.8**

Synonym: ***Nephroselmis gilva* Parke et Rayns**

Micrographs: Parke and Rayns, 1964; Figs. 16 - 21.
Moestrup, 1984; Figs. 2 - 7.
Thomsen, 1986; Figs. 36 - 38.

Present Findings.
Cells were found in samples from the Derwent River, Dru Point and Honey Moon Bay, as well as in a GSe/2 enrichment culture derived from the Derwent River and GSe enrichment cultures from Dru Point and Pipeclay Lagoon samples.

This is a new record for Australian waters.

Description.
Cells had two flagella which were approximately equal in length. These were seen on a dividing cell in culture (Fig. 5.4). Flagella were 7 μm in length on one cell, and 18 μm on the other cell, presumably the parent. "Spider-web" scales were seen on both the cell body and the flagella. These scales consisted of unevenly-spaced and irregular concentric rings crossed by radiating ribs; their structure was seen more clearly in uranyl acetate material (Fig. 5.5) than in shadow cast samples (Fig. 5.8).

Body scales were of two different types; one type, 0.22 - 0.33 μm ($\bar{x}$=0.24 μm; n=7), was larger than the other, 0.13 - 0.17 μm ($\bar{x}$=0.15 μm; n=7), and appeared to have a greater number of concentric rings. Due to sample preparation technique, patterns on the different scales could not be distinguished, but the size difference was clearly visible (Fig. 5.7).

Flagellar scales had a short spine arising from the scale centre, usually directed towards the flagellar tip, and extending beyond the scale rim (Figs. 5.6, 5.7). Scales were 0.16 - 0.25 μm ($\bar{x}$=0.20 μm; n=7), with an average spine length of 0.18 μm (n=7). Fine hair scales, approximately 0.5 μm in length and slightly curved, were also found on the flagella, but were more often detached.

Scale dimensions of wild and cultured cells are compared with literature records in Table 5.5; no significant differences were found.

Distribution.

This species has been reported from both temperate and tropical coastal waters including those of the UK, Denmark, Thailand and New Zealand (Moestrup, 1984; and references therein).

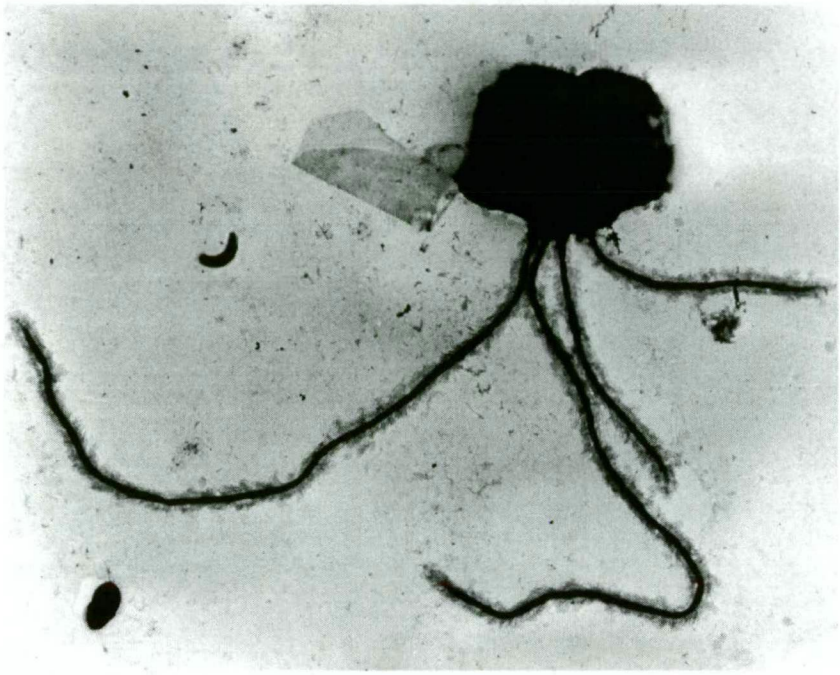


Fig. 5.4: *M. gilva* dividing cells; from Dru Point

(Micrograph no: 4876)

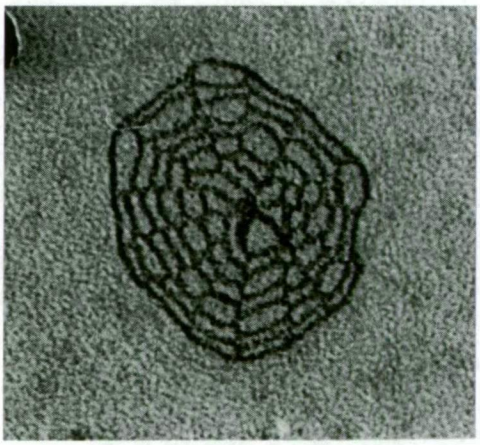


Fig. 5.5: *M. gilva* "spider-web" body scale (0.2 μm), uranyl acetate stained; from the Derwent River

(Micrograph no: 5165)

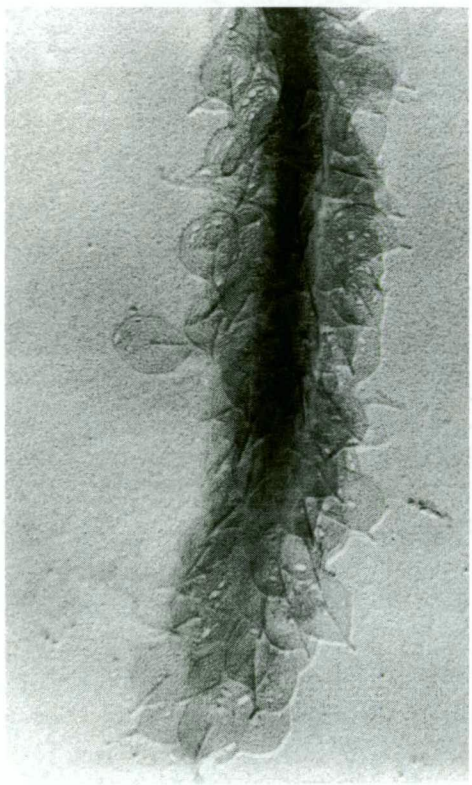


Fig. 5.6: *M. gilva* flagellar detail, showing "spider-web" scales (0.2 μm) with central spine; from Dru Point

(Micrograph no: 4875)

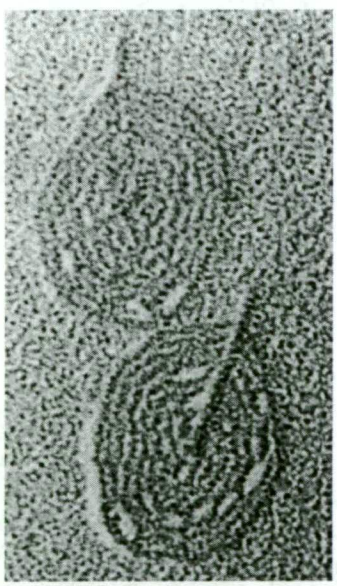


Fig. 5.7: Flagellar scales of *M. gilva* (0.2 μm) with central spine; from Dru Point

(Micrograph no: 4877)

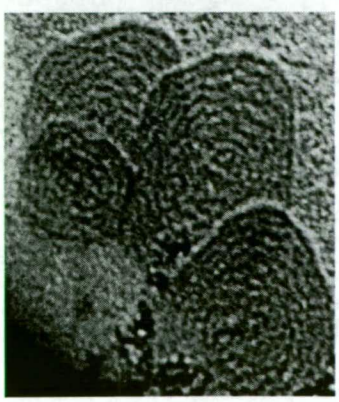


Fig. 5.8: Body scales of *M. gilva*, showing two different sizes (0.25 μm and 0.15 μm); from Dru Point

(Micrograph no: 4887)

Table 5.5: *Mamiella gilva* scales from different sources.

SOURCE	CELL BODY SCALES		FLAGELLAR SCALES	
	Large Scales (μm)	*Small Scales* (μm)	*Dimensions* (μm)	*Spine Length* (μm)
UK (Parke & Rayns, 1964)	0.23	ND	0.22	0.2
Thailand, Denmark, and New Zealand (Moestrup, 1984)	0.22 (n=4)	0.16 (n=3)	0.22 - 0.24 ($\bar{x}$ =0.23; n=4)	0.16 - 0.17 ($\bar{x}$ =0.17; n=3)
Tasmania, Australia - cultured cells	0.29 - 0.33 ($\bar{x}$ =0.31; n=2)	ND	0.18 - 0.25 ($\bar{x}$ =0.21; n=12)	0.14 - 0.21 ($\bar{x}$ =0.18; n=5)
- wild material	0.20 - 0.25 ($\bar{x}$ =0.22; n=5)	0.13 - 0.17 ($\bar{x}$ =0.15; n=7)	0.16 - 0.19 ($\bar{x}$ =0.18; n=2)	0.18 ($\bar{x}$ =0.18; n=2)

ND = Not determined

Measurements were made of cultured cells unless otherwise stated.

Mantoniella squamata **(Manton et Parke) Desikachary** **Figs. 5.9 - 5.11**

Micrographs: Manton and Parke, 1960; Figs. 1 - 8.
Barlow and Cattolico, 1980; Figs. 2 - 5.
Hallegraeff, 1983; Fig. 25.
Thomsen, 1986; Figs. 34 - 35.
Marchant et al, 1989; Figs. 1 - 3, 7.

Present Findings.
Cells were seen in samples from Oyster Cove Point, Fleurty Point, Deep Bay, Roches Beach and Pipeclay Lagoon. Scales were also found in samples from the Derwent River and Southport.

Description.
Mantoniella squamata has two flagella of unequal length, the shorter one being indistinguishable under the light microscope and only seen in fortuitously orientated material under the electron microscope (Barlow and Cattolico, 1980). Only the longer flagellum, 8 - 15 μm in length ($\bar{x}$=10; n=4), was seen in the present samples (Fig. 5.9).

Both the flagella and the cell body were covered with small "spider-web" scales, consisting of a series of unevenly-spaced and irregular concentric rings, with eight evenly-spaced straight ribs radiating from the centre (Fig. 5.11). Both cell and flagellar scales are morphologically similar, but those on the cell body are slightly larger than those on the flagella (Barlow and Cattolico, 1980; Marchant et al, 1989). In the Tasmanian material, body scales were usually detached from the cell, although flagellar scales remained on long flagellum (Figs. 5.9, 5.10). Insufficient body scales were found for size comparison.

Hair scales were also seen on the long flagellum (Fig. 5.10); these were generally 0.5 μm long and often slightly curved, in agreement with observations made by Manton and Parke (1960) and Barlow and Cattolico (1980). However, the tuft of three 1.0 μm hair scales at the distal tip of the long flagellum, reported by these authors, was not observed in the Tasmanian material. These hair scales may have become detached during sample preparation.

The Tasmanian specimens were similar in both size and scale structure to the type species (Manton and Parke, 1960), and closely matched material from the East Current Australia described by Hallegraeff (1983). Cells from Australian waters were

generally smaller than those from other samples (Table 5.6), while scale structure was similar regardless of location. Scales of *M. squamata* from Antarctica had small central knobs and seven less evenly-spaced, instead of eight evenly-spaced, radiating ribs (Marchant et al, 1989).

Distribution.

M. squamata has been reported from temperate and polar, coastal and oceanic waters in both hemispheres, including those of the Arctic and Antarctic, England, Greenland, Finland, Norway, the Western Baltic Sea, the North Atlantic Ocean, Thailand and Australia (Estep et al, 1984, and references therein; Thomsen 1986; Jochem, 1990).

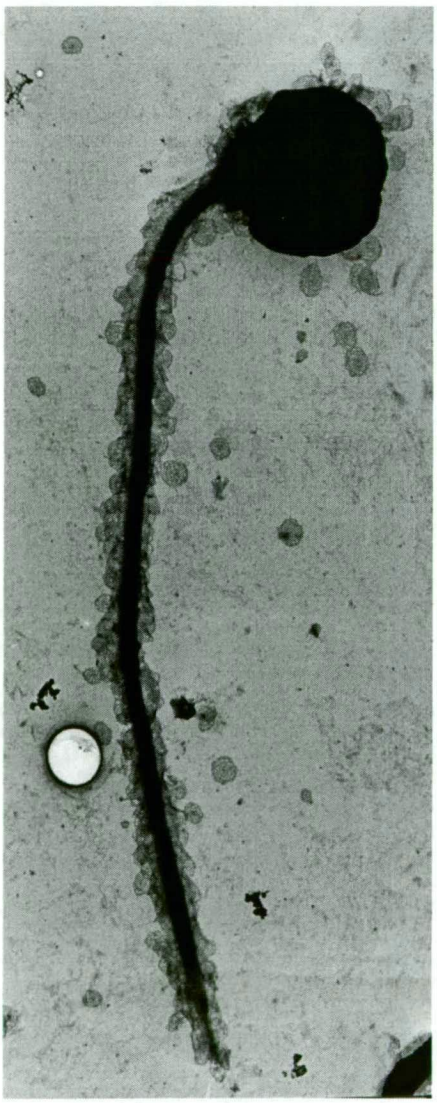


Fig. 5.9: *M. squamata* cell (c. 2 μm); flagellum (c. 10 μm) with "spider-web" scales; from Fleurty Point

(Micrograph no: 5542)

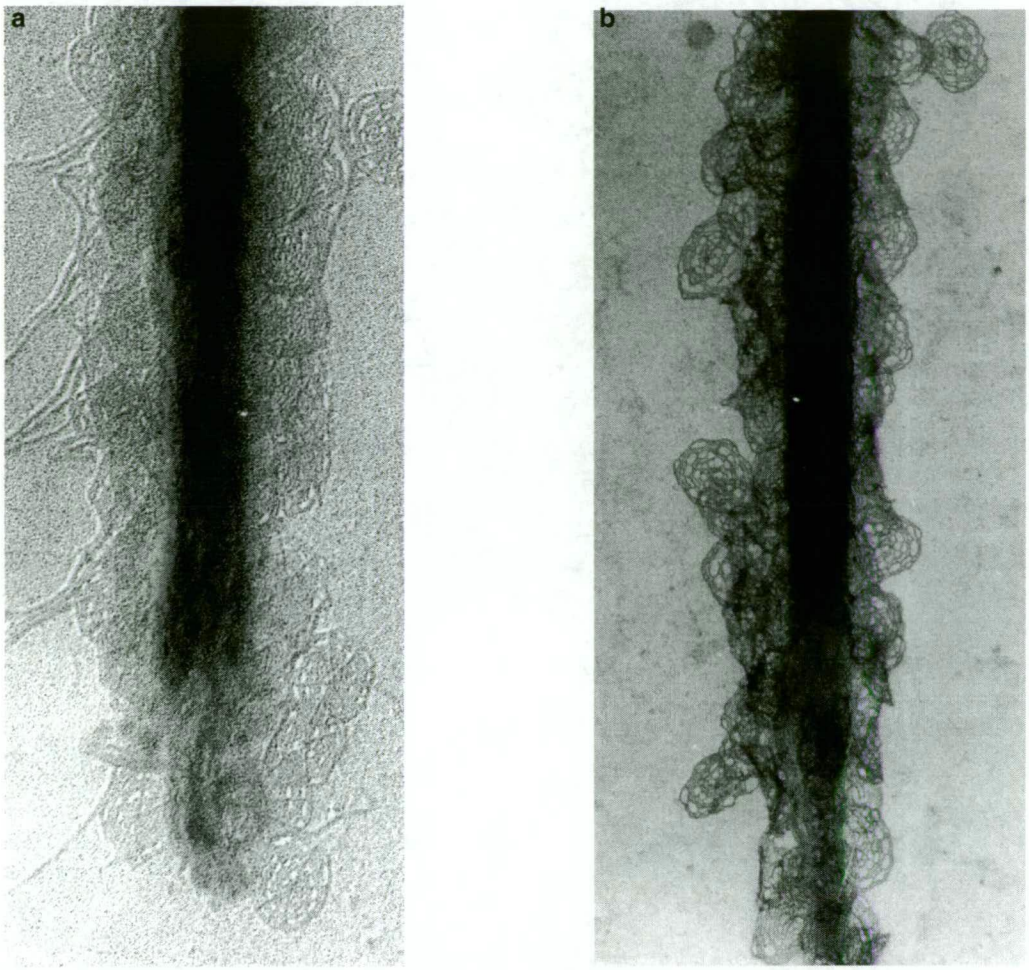


Fig. 5.10: *M. squamata* flagellar detail, showing "spider-web" scales (c. 0.2 μm) and flagellar hairs (0.5 μm); (a) shadow cast, and (b) uranyl acetate stained material; from Oyster Cove Point

(Micrograph no: 4944, 5542)

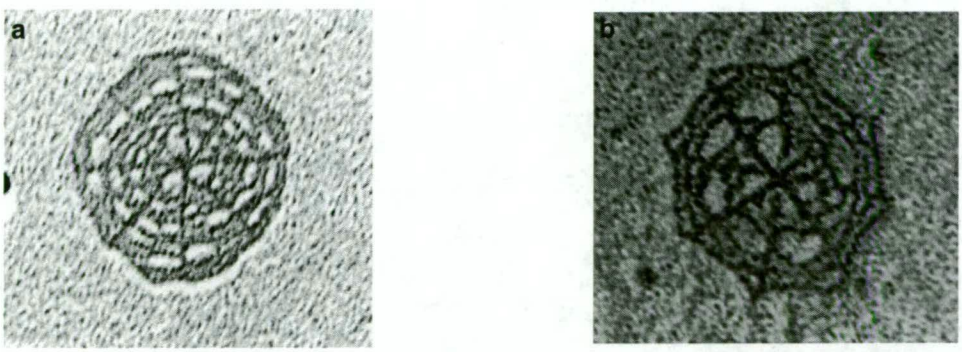


Fig. 5.11: Detail of *M. squamata* "spider-web" scales (c. 0.2 μm) showing 8 radiating ribs and concentric rings; (a) shadow cast, and (b) uranyl acetate stained material; from Roches Beach and Pipeclay Lagoon

(Micrograph no: 5035, 5203)

Table 5.6: *Mantoniella squamata* from different locations.

SOURCE	CELL SIZE	FLAGELLA	BODY SCALES	FLAGELLAR SCALES
	(μm)	*Length (μm)*	*Diameter (μm)*	*Diameter (μm)*
UK *(type)* (Manton & Parke, 1960)	3 - 5	9 - 17.5	0.15 - 0.40	
UK (Barlow & Cattolico, 1980)	2.4 - 4.0	6 - 16	0.10 - 0.40	
Denmark (Marchant et al, 1989)	1.7 - 3.3 ($\bar{x}$ =2.3; n=16)	5.9 - 13 ($\bar{x}$ =8.9; n=18)	0.22 - 0.33 ($\bar{x}$ =0.27; n=67)	0.20 - 0.30 ($\bar{x}$ =0.24; n=176)
Greenland (Marchant et al, 1989)	-	-	0.25 - 0.30 ($\bar{x}$ =0.27)	0.22 - 0.27 ($\bar{x}$ =0.24)
Antarctica (Marchant et al, 1989)	2.7 - 3.5	10 - 30 ($\bar{x}$ =22; n=25)	0.14 - 0.33 ($\bar{x}$ =0.24; n=172)	
East Australian Current (Hallegraeff, 1983)	1 - 3	6 - 9	0.16 - 0.22	
Tasmania, Australia	2.0 - 2.5 ($\bar{x}$ =2.2; n=5)	8 - 15 ($\bar{x}$ =10; n=4)	0.17 - 0.27 ($\bar{x}$ =0.20; n=12)	

***Pyramimonas amylifera* Conrad** **Figs. 5.12 - 5.13**

Micrographs: Manton et al, 1963; Figs. 36 - 41.
Norris and Pienaar, 1978; Fig. 3.
Inouye and Horiguchi, 1982; Figs. 2 - 5.
McFadden et al, 1986; Fig. 10A - L.

Present Findings.
Scales were seen in samples from Southport and Honey Moon Bay.

Description.
Pyramimonas amylifera cells were observed under the light microscope in samples before fixation. They were distinguished by their large size (c. 15 x 10 μm) and their eight flagella. Under the transmission electron microscope, two types of box scales were positively identified as belonging to *P. amylifera.*

The first type of scale was a square "basket-like" scale. The base was divided into eight equal segments with a central ring of eight apertures and an outer margin of sixteen smaller and more irregular apertures. Eight outer rods, perpendicular to the base, were distally connected by four "cross-bars" forming an upper peripheral square (Fig. 5.12). Scales were 0.24 - 0.27 μm in size ($\bar{x}$=0.25 μm; n=22), with a height of 0.06 - 0.08 μm ($\bar{x}$=0.07; n=19).

This agreed with previous descriptions of this species from Australian waters (McFadden et al, 1986), as well as from South Africa and Japan (Norris and Pienaar, 1978; Inouye and Horiguchi, 1982). Another type of base patterning has been described for *P. amylifera* (North European/North American form) with an intermediate ring of smaller apertures (Moestrup et al, 1987; Fig. 50).

The second type of scale was also square, 0.25 - 0.27 μm in size ($\bar{x}$=0.26; n=7), and had eight irregular apertures arranged around a central knob. Numerous small protrusions were evenly spaced around the edge of the scale (Fig. 5.13). This form is again representative of *P. amylifera* scales from South Africa, Japan and Australia, with a different form of body scale reported from Europe and North America (Moestrup et al, 1987; Fig. 50).

Distribution.

P. amylifera is a commonly reported species with records from Belgium, UK, Norway, USA, Canada, South Africa, Japan and Australia (McFadden et al, 1986, and references therein; Smith and Hobson, 1994).

Fig. 5.12: *P. amylifera* "basket-like" body scale (0.25 μm); from Southport

(Micrograph no: 5394)

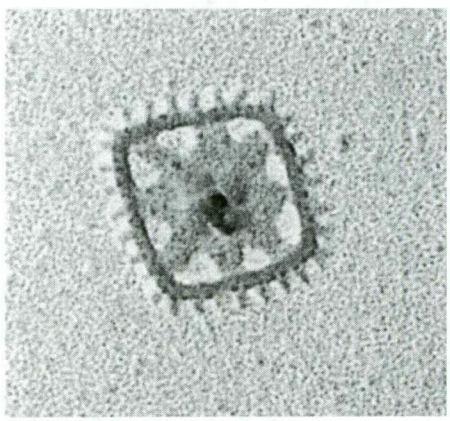


Fig. 5.13: *P. amylifera* square body scale (0.25 μm); from Southport

(Micrograph no: 5395)

***Pyramimonas cirolanae* Pennick** **Figs. 5.14 - 5.15**

Micrographs: Pennick, 1982a; Figs. 8 - 9.
McFadden et al, 1986; Fig. 8K - Q.

Present Findings.
Scales were observed in samples from the Derwent River and Pipeclay Lagoon.

Pyramimonas cirolanae grew in GSe enrichment cultures derived from Derwent River and Deep Bay samples.

Description.
Cells were 4.0 - 4.5 μm in length, with four flagella, each approximately 8 μm long (Fig. 5.14). There are six types of scales: three types are found on the flagella and three types on the cell body (Pennick, 1982a).

The most distinctive scale type were box scales, found on the cell body. Scales were divided into quarters by four bars at right angles to each other and joined at the centre. Each quarter had 6 - 7 irregular apertures, usually with one long aperture present alongside each of the four central bars (Fig. 5.15). One side of the scale appeared dentate, as noted by McFadden et al (1986). Scale size ranged from 0.22 - 0.26 μm ($\bar{x}$ = 0.24 μm; n=6), which agreed with the size given in the original species description (Pennick, 1982a). There was no difference in the size or structure of this scale type, regardless of whether it was from wild samples or cultured material.

Other scale types were more difficult to distinguish. A few crown scales were occasionally observed at the cell periphery; it was likely that the remainder had become detached during sample preparation. Limuloid scales and hair scales were seen on the flagella. Hair scales were approximately 1 μm in length and had a thread-like distal tip, c. 0.3 μm (Fig. 5.14). Small pentagonal and box scales, usually found on the flagella and cell body respectively, were not observed.

P. cirolanae also has trichocysts (Pennick, 1982a). Broken tubular pieces of discharged trichocyts were sometimes seen from cells grown in culture (Fig. 5.14).

Distribution.

P. cirolanae has been previously reported from the UK, Norway and the Barents Sea in the northern hemisphere, and south-east Australia in the southern hemisphere (McFadden et al, 1986; and references therein). Blooms of *P. cirolanae* and *P. disomata,* with combined cell densities of over 3 x 10^8 cells mL^{-1}, have been recorded from brackish waters in south-east Australia (McFadden et al, 1986), but these blooms did not have any harmful effects.

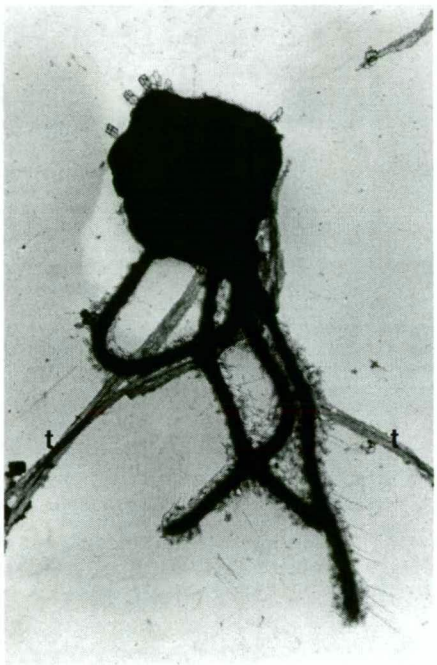


Fig. 5.14: *P. cirolanae* cell (4 μm) with four flagella (c. 8 μm) and trichocysts (t); from a Deep Bay enrichment culture

(Micrograph no: 5142)

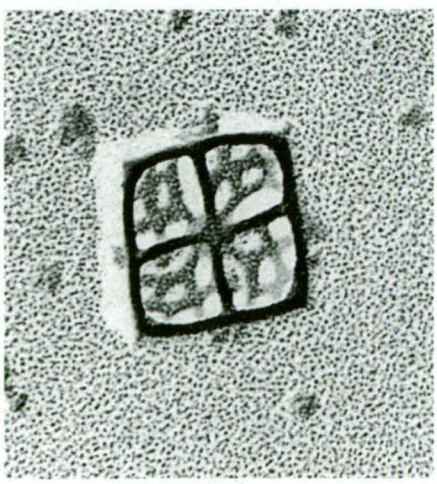


Fig. 5.15: *P. cirolanae* box scale (0.25 μm) detached from cell body; from Deep Bay

(Micrograph no: 5137)

***Pyramimonas grossii* (Parke) Manton** **Figs. 5.16 - 5.22**

Micrographs: Manton et al, 1963; Figs. 29 - 34.
Leadbeater, 1972b; Figs. 12 - 13.
Pennick and Clarke, 1976; Plate 58.
Hallegraeff, 1983; Fig. 27.
McFadden et al, 1986; Fig. 8A - I.

Present Findings.
Cells or scales were found in most samples, namely those from the Derwent River, Storm Bay, Dru Point, Oyster Cove Point, Fleurty Point, Deep Bay, Southport, Pipeclay Lagoon, Roches Beach, Eaglehawk Neck, Honey Moon Bay and Little Swanport

P. grossii was common in enrichment cultures and grew well in cultures established from the following samples: Derwent River and Storm Bay (GSe, GSe/2, ML media) Dru Point (GSe/2 and GSe/10 media), Deep Bay (GSe/2 and GSe media), Southport (GSe medium), Honey Moon Bay (GSe medium), Little Swanport (GSe/10 and ML media) and Maria Island (ML medium).

A unialgal culture of *P. grossii* was obtained by micro-pipetting, and is currently maintained in the CSIRO Living Collection of Microalgae (CS-489) in GSe medium at 15°C under standard growth conditions.

Description.
Cells were 5 - 3 x 4 - 3 μm ($\bar{x}$=3.9 x 3.4 μm; n=7), slightly smaller than the sizes reported in the literature (Table 5.7). Each cell had four flagella, 6 - 9 μm in length, (Fig. 5.16).

Six types of scales have been described for *P. grossii* (Pennick and Clarke, 1976), and all were observed in the present study.

The most distinctive scale type is the medium-sized box scale and this was used as the main identifying feature for *P. grossii*. There was a characteristic octagonal pattern on the scale base, including a central ring of 8 oval perforations, an intermediate square of 8 - 9 alternate oval and elongate perforations, and an outer square of approximately 20 oval perforations (Fig. 5.18). The scale margin appeared smooth when viewed from one side and dentate when viewed from the other.

This scale type is usually found on the cell body, but scales often became detached during sample preparation.

Small box scales (Fig. 5.19) and large "crown" scales (Fig. 5.17) were the two other scale types usually seen on the cell body (Pennick and Clarke, 1976), but again were more often found detached from the cell.

Limuloid and hair scales were seen on the flagella (Figs. 5.16, 5.20). The limuloid scales, 0.26 - 0.33 μm ($\bar{x}$=0.3 μm; n=6), had a central spine which ran the length of the scale and protruded from one end. Two apertures were found near the periphery of the scale, at the opposite end from the protruding spine (Fig. 5.21). Hair scales were 0.9 - 1.05 μm in length ($\bar{x}$=0.95 μm; n=11), and had a pointed tip (Fig. 5.20). These scales have distinct structures which have been found to be useful taxonomic features but can only be observed with specific staining (Marin and Melkonian, 1994).

The third type of flagellar scale was a small pentagonal scale, 0.03 - 0.04 μm ($\bar{x}$=0.04 μm; n=21) with a central knob (Fig. 5.22). These scales form an inner layer on the flagella (Pennick and Clarke, 1976) but were only observed in samples when detached.

Scale details, especially of the limuloid and hair scales, were seen more clearly in uranyl acetate stained material in comparison to shadow cast samples. There was no apparent difference in scale structure of wild or cultured material. Scale sizes were generally in agreement with other observations (Table 5.7).

P. grossii has up to four trichocysts which, on discharge, cause the cell to "jump" in a characteristic fashion. Broken tubular pieces of discharged trichocysts were occasionally observed in samples.

Distribution.

P. grossii is common in temperate coastal waters and has been reported from Denmark, Norway, UK, the Mediterranean Sea, Japan, Canada, New Zealand and Australia (McFadden et al, 1986, and references therein; Smith and Hobson, 1994).

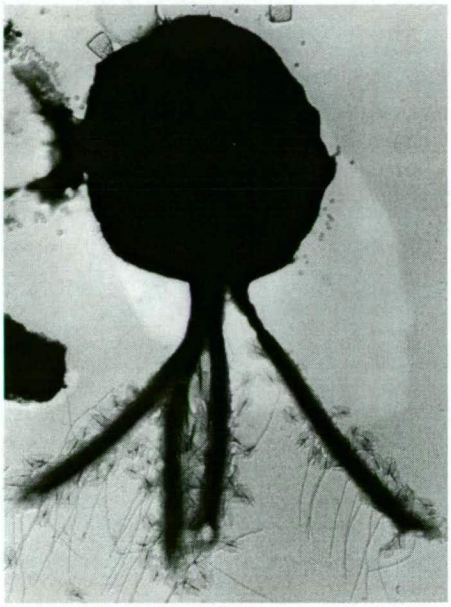


Fig. 5.16: *P. grossii* cell (c. 4 μm); from Deep Bay

(Micrograph no: 5000)

Fig. 5.17: *P. grossii* "crown" scales (c. 0.25 μm); from a Derwent River enrichment culture

(Micrograph no: 4747, 4748)

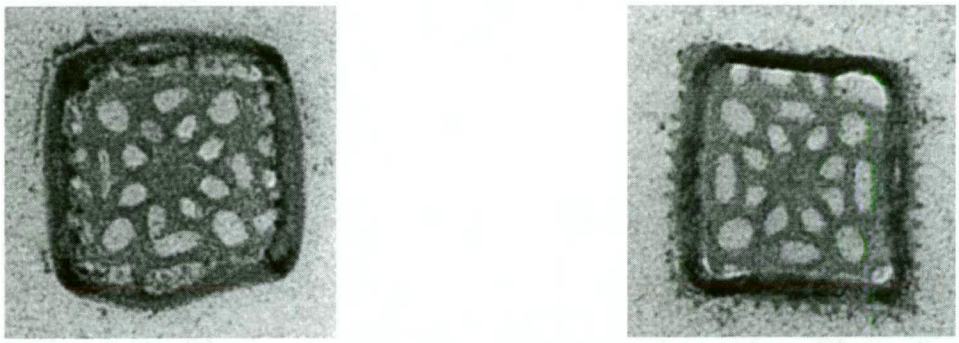


Fig. 5.18: *P. grossii* box scales (0.25 μm); from a Derwent River enrichment culture

(Micrograph no: 4745)

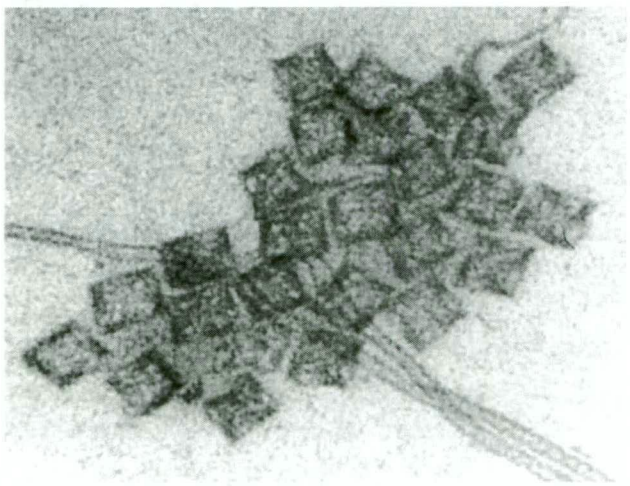


Fig. 5.19: *P. grossii* small body scales (0.04 μm); from a Derwent River enrichment culture

(Micrograph no: 4743)

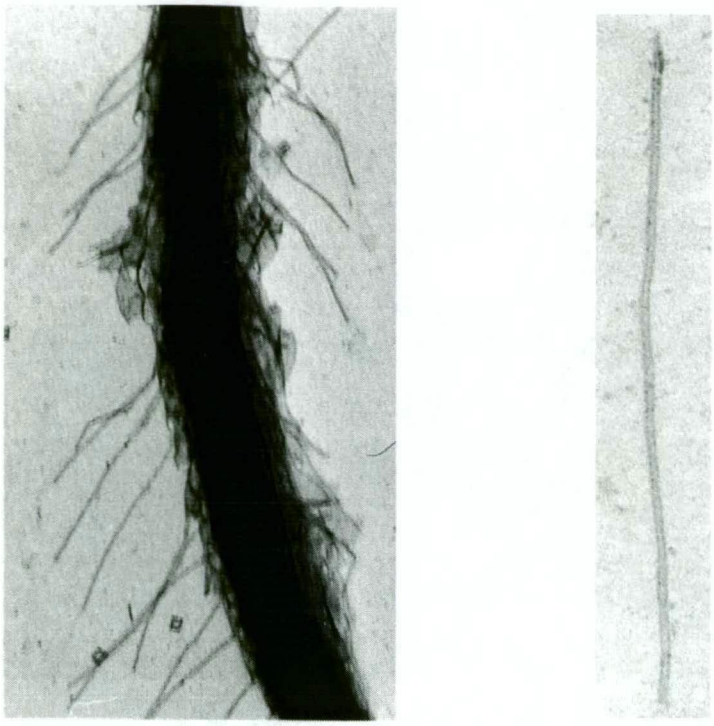


Fig. 5.20: *P. grossii* flagellar hair scales (0.9 - 1.05 μm); from a Derwent enrichment culture

(Micrograph no: 4736, 4737)

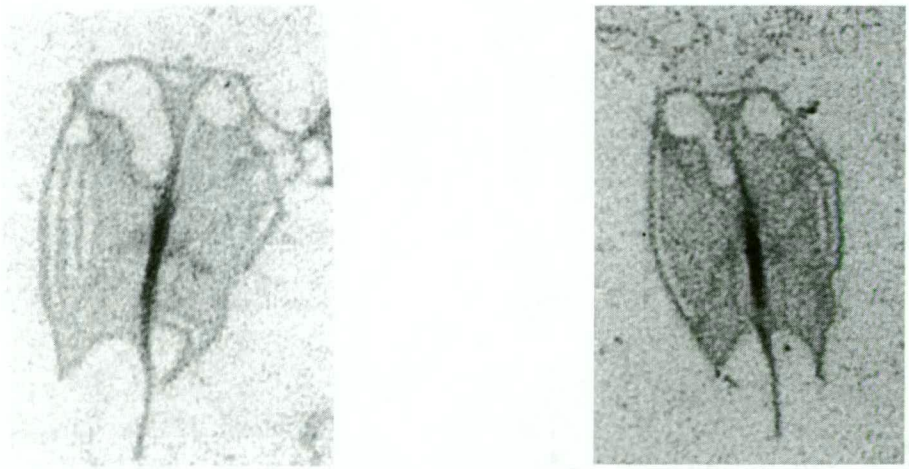


Fig. 5.21: *P. grossii* limuloid scales (c. 0.3 μm); from Derwent enrichment cultures

(Micrograph no: 4736, 4774)

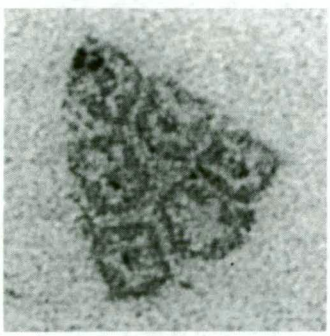


Fig. 5.22: *P. grossii* pentagonal flagellar scales (0.04 μm); from a Derwent enrichment culture

(Micrograph no: 4743)

Table 5.7: Comparison of *P. grossii* cell and scale dimensions.

SOURCE	MATERIAL EXAMINED	CELL SIZE (μm)	FLAGELLA LENGTH (μm)	BODY SCALE DIMENSIONS			FLAGELLAR SCALE DIMENSIONS		
				Crown	*Box* (μm)	*Sml. Box*	*Hair*	*Limuloid* (μm)	*Pentagon*
UK (Manton et al, 1963)	Culture	6 x 5	6	0.3 (n=2)	0.3 (n=5)	0.05 (n=8)	0.95 (n=1)	0.34 (n=3)	0.04 (n=1)
North Sea (Pennick & Clarke, 1976)	Culture	8 - 6 x 6 - 5	= cell length	0.22 x 0.15	0.25	0.045	ND	0.35	0.035 - 0.04
Norway (Leadbeater, 1972b)	Culture	c. 3	6	-	0.23 - 0.24 (n=2)	-	-	-	-
New South Wales, Australia (Hallegraeff, 1983)	Wild	-	-	0.30 - 0.38	0.24 - 0.28	-	-	-	-
Victoria, Australia (McFadden et al, 1986)	Culture	8 - 4 x 6 - 4	≥ cell length	-	0.25 (n=3)	-	-	0.20 (n=1)	
Tasmania, Australia	Wild	3 - 2.5 x 4 - 3.5 (n=1)	6 - 7 (n=1)	-	0.24 - 0.26 ($\bar{x}$ =0.24; n=7)	0.04 (n=4)	0.90 - 0.98 ($\bar{x}$ =0.95; n=3)	0.30 (n=1)	0.03 (n=1)
	Culture	5 - 3 x 4 - 3 (n=5)	6 - 9 (n=5)	0.24 - 0.28 ($\bar{x}$ =0.26; n=5)	0.22 - 0.26 ($\bar{x}$ =0.25; n=14)	0.04 - 0.05 ($\bar{x}$ =0.04; n=28)	0.95 - 1.05 ($\bar{x}$ =0.94; n=8)	0.26 - 0.33 ($\bar{x}$ =0.29; n=5)	0.03 - 0.04 ($\bar{x}$ =0.04; n=21)

***Pyramimonas longicauda* (Van Meel) Inouye et Chihara** **Figs. 5.23 - 5.25**

Micrographs: Inouye et al, 1984; Fig. 6.
McFadden et al, 1986; Fig. 11A - F.

Present Findings.
Two scale types were found in samples from Pipeclay Lagoon, the Derwent River and Oyster Cove Point.

Description.
The first scale type was a "basket-like" scale, 0.81 - 0.85 μm ($\bar{x}$=0.85 μm; n=5). The centre "basket" section of the scale was patterned with numerous regular perforations, and attached to a proximal base plate by 10 or 12 short supporting struts. The base plate had a marginal pattern of larger apertures and a dentate rim (Figs. 5.23, 5.24). This description agreed with that given by Inouye et al (1984) and by McFadden et al (1986), with the only difference being a constant number of 12 supporting struts. These scales are found in an intermediate layer on the cell body.

The second scale type was also "basket-like", but slightly larger than the previous scale type, having a base of c. 1 μm (n=2), with a more open structure (Fig. 5.25). Eight upright rods extended from the corners and central points of the base and were distally connected by peripheral arches. Small spines were found at the distal ends of the four corner rods. The base was patterned with numerous irregular apertures. This scale type also agreed with the material described by Inouye et al (1984) and McFadden et al (1986).

Distribution.
P. longicauda has been previously reported only from Belgium, Japan and south-east Australia (McFadden et al, 1986; and references therein).

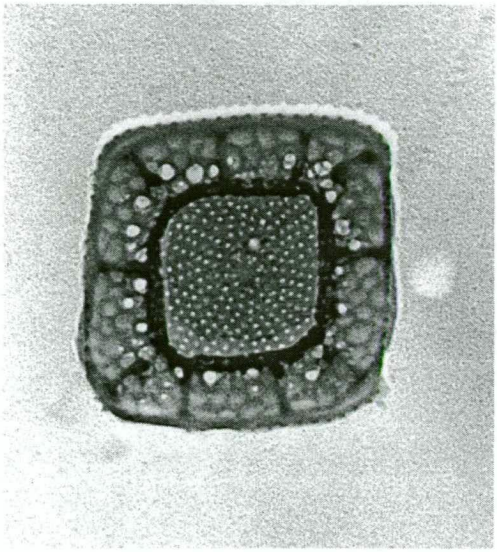


Fig. 5.23: *P. longicauda* "basket" scale (0.85 μm) - distal view; from Oyster Cove Point

(Micrograph no: 4940)

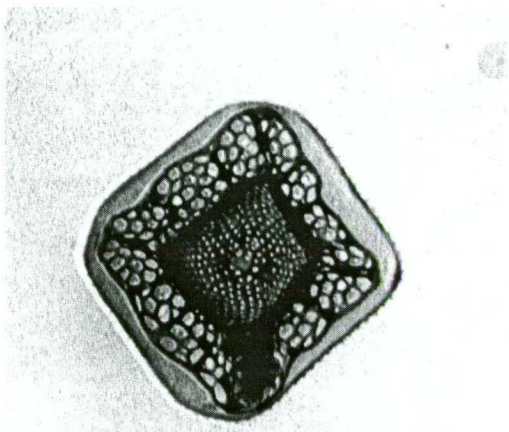


Fig. 5.24: *P. longicauda* "basket" scale (0.85 μm) - proximal view; from Pipeclay Lagoon

(Micrograph no: 5014)

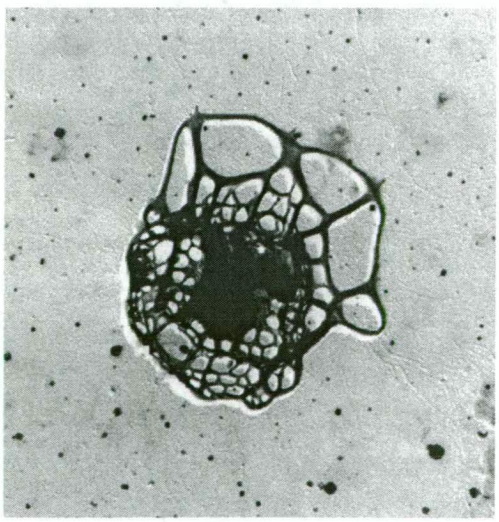


Fig. 5.25: *P. longicauda* open "basket" scale (c. 1 μm); from the Derwent River

(Micrograph no: 5336)

***Pyramimonas obovata* Carter** **Figs. 5.26 - 5.27**

Micrographs: Pennick et al, 1976; Plates 40 - 41.
Hallegraeff, 1983; Fig. 28
McFadden et al, 1986; Fig. 4.

Present Findings.
Scales were observed in samples from Pipeclay Lagoon, Dru Point Honey Moon Bay and Storm Bay.

Description.
These square box scales, 0.24 - 0.29 µm ($\bar{x}$ = 0.26 µm; n=6), had a pattern of raised ridges forming at least two central squares, one within the other (Fig. 5.27). A few scales appeared to have a central knob (Fig. 5.26). Scales were slightly smaller than those reported from the East Australian Current, which were 0.28 - 0.36 µm (Hallegraeff, 1983).

McFadden et al (1986) have suggested that *P. obovata* and *P. disomata* are synonyms for the same species. However, according to Pennick (1984), the box scales of these two species have very different patterns. In addition, Norris and Pienaar (1978) reported another species, *P.* aff. *plurioculata*, with box scales patterned with one distinctive square ridge on the base and a central knob. Further investigation of these three species, comparing wild material and cultured cells, is required to clearly define scale morphology and to clarify taxonomy.

Distribution.
P. obovata has been recorded from coastal and oceanic waters, mostly in temperate regions, and has been reported from Denmark, USA (Washington state), UK, Greenland, the North Atlantic Ocean and the East Australian Current (Estep et al, 1984 and references therein).

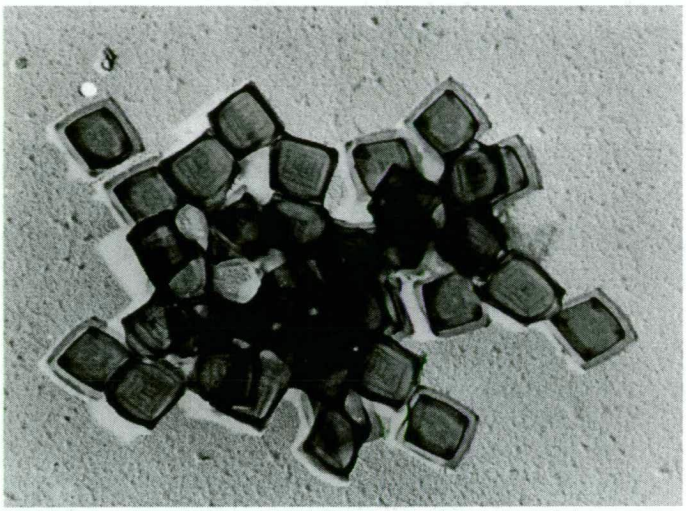


Fig. 5.26: *P. obovata* field of scales; from Honey Moon Bay

(Micrograph no: 5348)

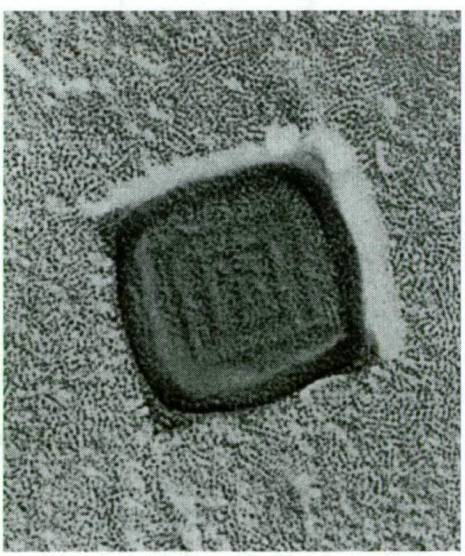


Fig. 5.27: *P. obovata* box scale (c. 25 μm); from Dru Point

(Micrograph no: 5014)

***Pyramimonas virginica* Pennick** **Figs. 5.28 - 5.29**

Micrographs: Pennick, 1977; Figs. 11 - 16.
McFadden et al, 1986; Fig. 12.
Thomsen, 1986; Fig. 43 - 45.

Present Findings.
Pyramimonas virginica was found in samples from Oyster Cove Point, Fleurty Point, Deep Bay and Roches Beach.

Description.
P. virginica is one of the smallest species of *Pyramimonas*. In this survey, cells were 1.9 - 2.3 x 1.5 - 2.0 μm in size ($\bar{x}$=2.1 x 1.8 μm; n=4). They were slightly smaller than the type species size of 2.7 - 3.5 x 1.9 - 2.4 μm (Pennick, 1977), but similar to cells recorded by McFadden et al (1986) from Victorian coastal waters, which were 2.0 - 3.5 x 2.0 μm.

There were four equal flagella, approximately the same length as the cell, with limuloid and hair scales (Fig. 5.28). Scale structures were not very clear due the shadow casting method used for these samples.

Only two types of scales were found on the cell body: small square scales, 0.07 μm, and larger hexagonal "basket-like" scales, approximately 0.21 μm in width and 0.23 μm in height. These larger scales had a hexagonal base with long, upright rods arising from each corner and connected distally by a single hexagonal rail to form the upper rim of the scale (Fig. 5.29). This description was slightly different to that given for scales of the type species, reported to have an upper rim consisting of two rails (Pennick 1977). However, observations made on other Australian material also show a single upper rail (McFadden et al, 1986) and it is possible that, like *P. amylifera*, there exists a southern form of this species. Further investigation of *P. virginica* from both northern and southern waters is required.

P. virginica has several trichocysts (McFadden et al, 1986), and discharged trichocysts were seen in some samples.

Distribution.

P. virginica was orginally described from Virginia, USA, and has since been reported from Greenland, Denmark, Thailand, New Zealand and south-east Australia, (Hori et al, 1995; and references therein), indicating that it has a cosmopolitan distribution.

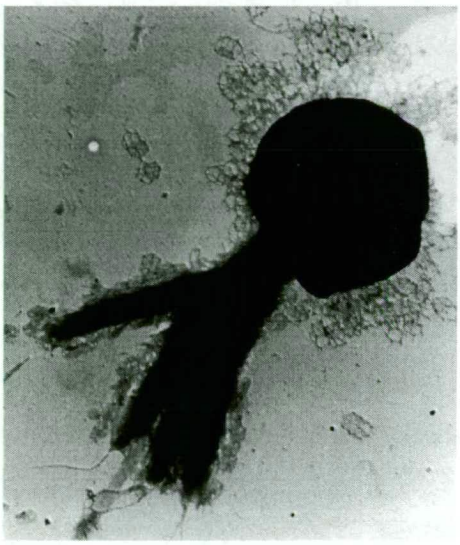


Fig. 5.28: *P. virginica* cell (c. 2 μm); from Pirates Bay

(Micrograph no: 5554)

Fig. 5.29: *P. virginica* "basket" scales (0.2 μm); from Oyster Cove Point

(Micrograph no: 4935)

***Pyramimonas* sp. 1** **Figs. 5.30 - 5.32**

Present Findings.
Cells were found in Deep Bay, Southport, Pipeclay Lagoon and Coles Bay samples.

Description.
Cells were 4.0 - 5.3 x 3.4 - 4.8 μm (n=6) and had four flagella, ranging in length from 6 - 8 μm (Fig. 5.30). Like other *Pyramimonas* species, flagella became easily detached from the cell during sample preparation.

Two types of body scales were common: box scales, and crown scales (Fig. 5.32). Box scales, 0.29 - 0.32 μm ($\bar{x}$=0.29 μm; n=9), had a distinct central spine, c. 0.1 μm in height (n=2). Limuloid and hair scales were seen on the flagella (Fig. 5.31).

Only three *Pyramimonas* species have box scales with spines: *P. spinifera* Pennick, *P. moestrupii* McFadden and *P. olivacea* (Carter) McFadden. Each of these box scale types have distinct patterning on the scale base. Scales of *P. moestrupii* have numerous perforations on the base (McFadden et al, 1986), while those of *P. olivacea* are divided into eight equal segments by radiating ridges (McFadden et al, 1987). Pennick (1983) reported that scales of *P. spinifera* have a base that is divided into four sections, each with regular striations. No base patterning was seen in the Tasmanian material; it may have been possibly obscured by the thickness of the shadow casting metal.

It is probable that this is a new species of *Pyramimonas* but additional material is required to further study scale structure, using staining in preference to shadow casting.

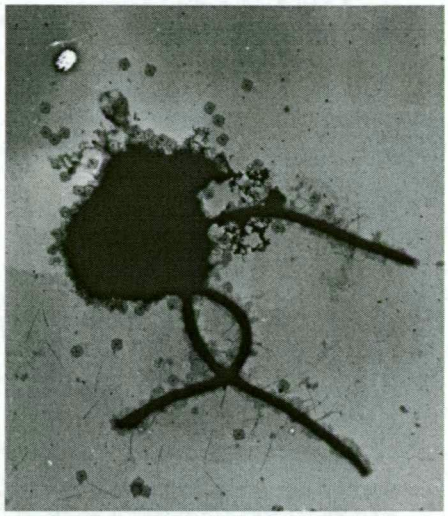


Fig. 5.30: *Pyramimonas* sp. 1, cell (c. 4 μm) showing scale covering, and missing one flagellum; from Southport

(Micrograph no: 5389)

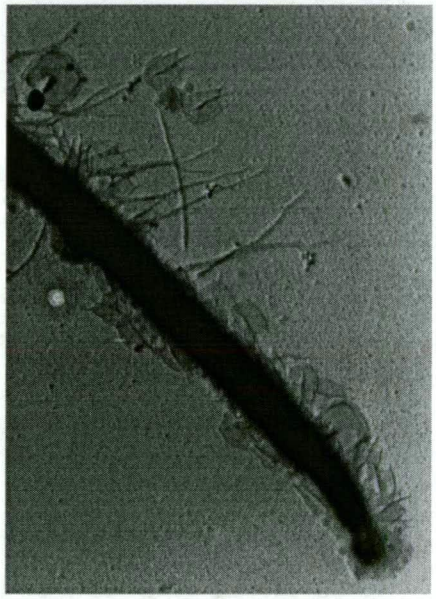


Fig. 5.31: *Pyramimonas* sp. 1, detail of flagellum, showing limuloid and hair scales; from Southport

(Micrograph no: 5391)

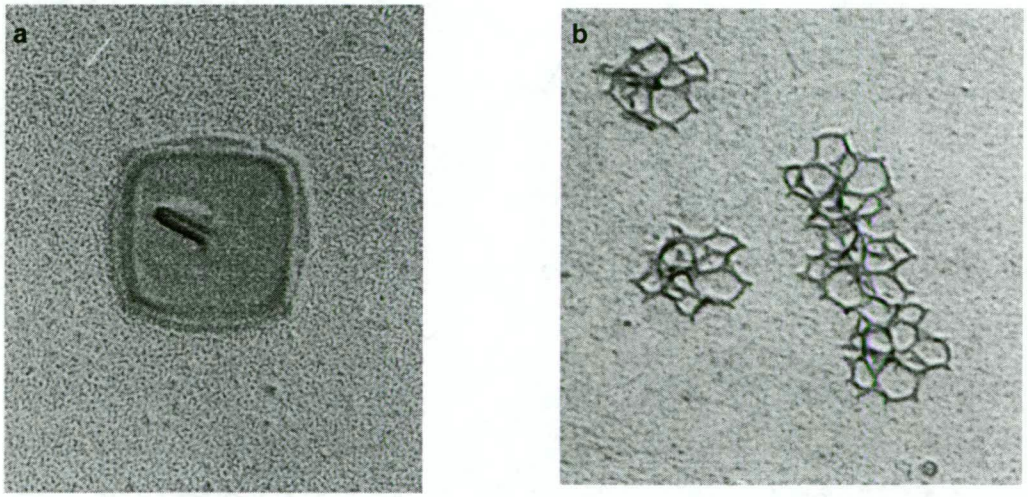


Fig. 5.32: *Pyramimonas* sp. 1, (a) spined box scales (c. 0.3 μm) and (b) crown scales (c. 0.3 μm); from Southport

(Micrograph no: 5390, 5574)

***Pyramimonas* sp. 2** **Figs. 5.33 - 5.34**

Present Findings.
Cells were found in Eaglehawk Neck and Simmons Point samples.

Description.
Cells were c. 4 μm (n=3) and had 4 flagella, which were slightly longer than the cell. c. 4.5 μm in length (Fig. 5.33).

Limuloid and hair scales were seen on the flagella, and box scales on the cell body. These box scales were 0.26 - 0.28 μm (n=6) and lacked distinct patterning (Fig. 5.34). Again, this may have been due to the shadow casting technique used.

At least one species of *Pyramimonas*, *P. orientalis* Butcher, is known to have box scales without obvious surface patterning (Pennick, 1984). This species has been recorded by Leadbeater (1972b; Figs. 10 - 11) from Norwegian coastal waters, and McFadden et al (1986; Fig. 7) from south-east Australia. In the latter case, faint longitudinal striations were seen on the scale base with freeze-etching.

Further investigation is required to identify this species.

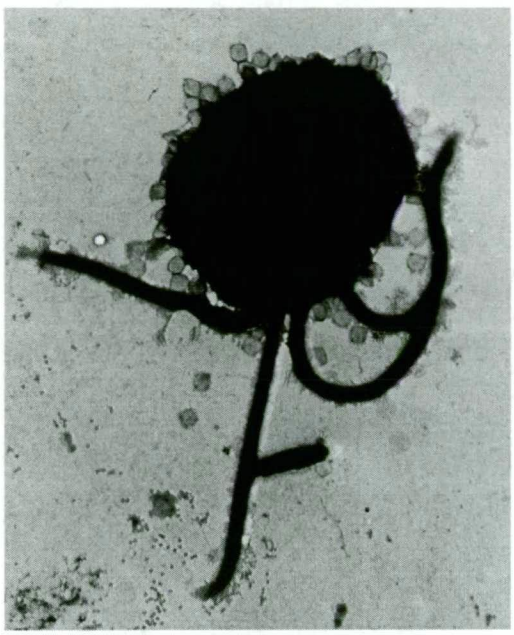


Fig. 5.33: *Pyramimonas* sp. 2, whole cell (c. 4 μm) with four flagella (c. 4.5 μm); from Simmons Point

(Micrograph no: 5506)

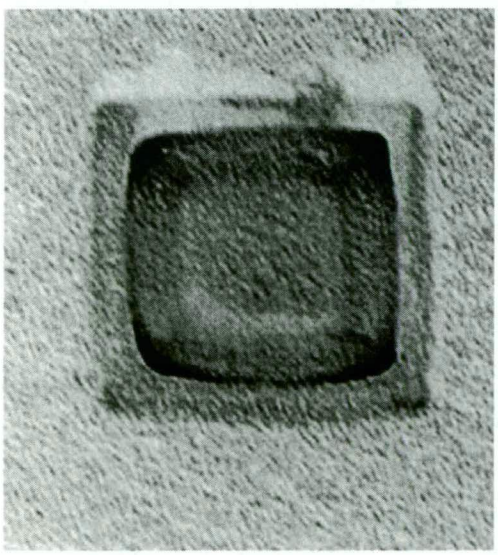


Fig. 5.34: *Pyramimonas* sp. 2, box scale (0.28 μm); from Simmons Point

(Micrograph no: 5508)

***Pyramimonas* sp. 3** **Fig. 5.35 - 5.38**

Present Findings.

Cells were found in samples from Pipeclay Lagoon, Roches Beach and Honey Moon Bay.

This species also grew in GSe enrichment cultures from the Derwent River, and in ML enrichments from Pipeclay Lagoon and Little Swanport. Unfortunately, unialgal isolations were unsuccessful.

Description.

Cells were 6 x 4 μm and had 4 flagella, 9 - 10 μm long (n=2).

Limuloid and hair scales were seen on the flagella, and crown and box scales were found on the cell body (Fig. 5.35). Small square body scales, c. 0.04 μm (n=10), were also seen (Fig. 5.37). Crown scales had an unusual eight-pointed structure (Fig 5.38), and were c. 0.25 μm in size (n=3). They looked somewhat similar to those of *P. disomata* (McFadden et al, 1986: Fig. 4C). Box scales, 0.18 - 0.20 μm ($\bar{x}$ =0.20 μm, n=6), had a central pattern of four perforations arranged around a central cross (Figs. 5.36, 5.37), which was a new type of patterning for *Pyramimonas* box scales.

This material probably represents a previously undescribed species of *Pyramimonas* but requires further study.

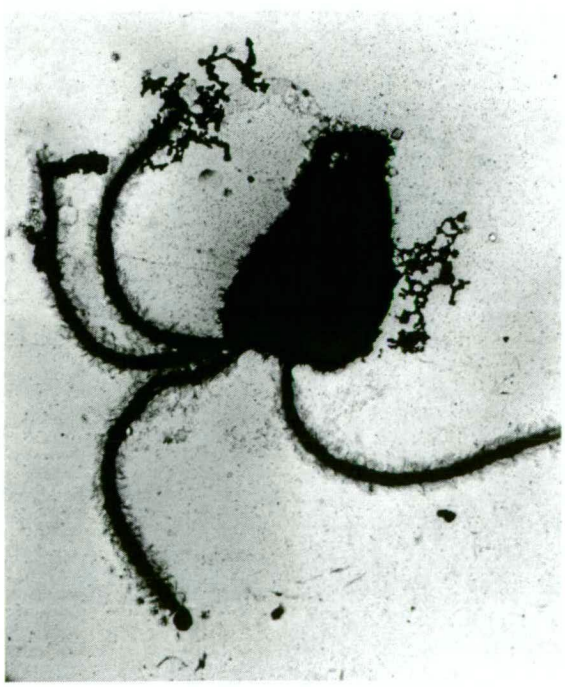


Fig. 5.35: *Pyramimonas* sp. 3, whole cell (6 x 4 μm) with four flagella (9 - 10 μm); from a Derwent enrichment culture

(Micrograph no: 5053)

Fig. 5.36: *Pyramimonas* sp. 3, box scale (0.2 μm) with four central perforations; from Pipeclay Lagoon

(Micrograph no: 5055)

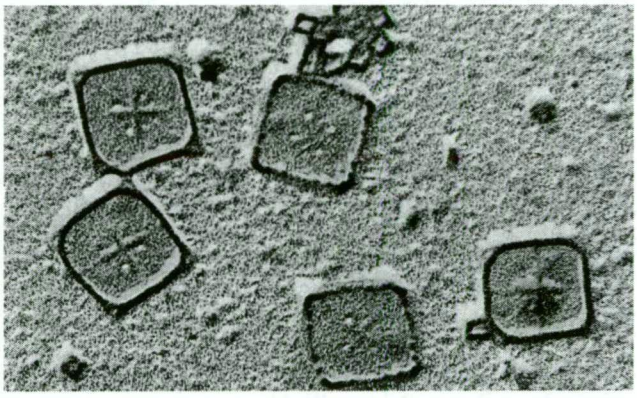


Fig. 5.37: *Pyramimonas* sp. 3, box scales and small square scales; from a Little Swanport enrichment culture

(Micrograph no: 5312)

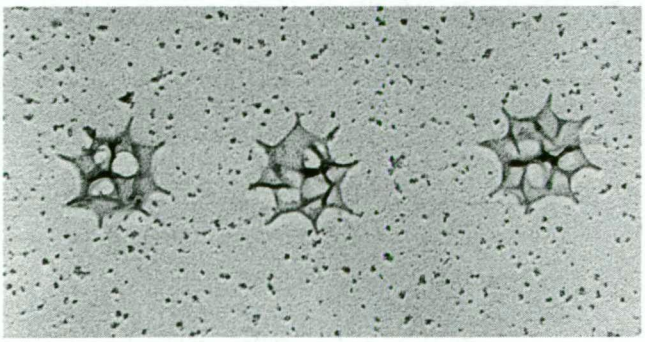


Fig. 5.38: *Pyramimonas* sp. 3, crown scales (c. 0.25 μm); from Pipeclay Lagoon

(Micrograph no: 5054)

Pyramimonas-like Box Scales — Figs. 5.39 - 5.43

There were five types of *Pyramimonas*-like box scales, detached from cells, that were seen at least once in various samples.

Pyramimonas sp. 4 had a box scale (Fig. 5.39) similar to that reported by McFadden et al (1986; Fig. 8J), with a structure intermediate between that of *P. grossii* and *P. cirolanae.*

Pyramimonas sp. 5 had a box scale (Fig. 5.40) which may possibly belong to *P. occidentalis*, given its distinctive central cross. However, it had a slightly scalloped rim, not shown in illustrations by Pennick (1982b, 1984).

Pyramimonas sp. 6 and 7 had box scales with central protrusions (Figs. 5.41, 5.42), and *Pyramimonas* sp. 8 had a scale type with an irregular perforated pattern (Fig. 5.43) not previously observed.

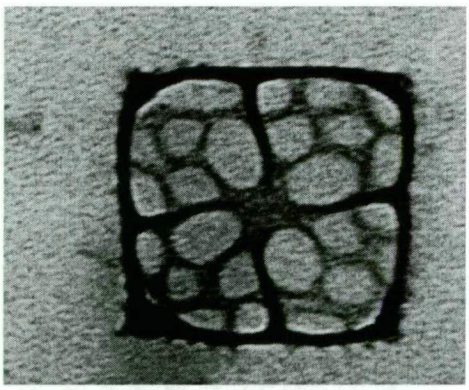


Fig. 5.39: *Pyramimonas* sp. 4, box scale (0.2 μm); from the Derwent River

(Micrograph no: 5167)

Fig. 5.40: *Pyramimonas* sp. 5, box scale (0.3 μm); from Pipeclay Lagoon

(Micrograph no: 5448)

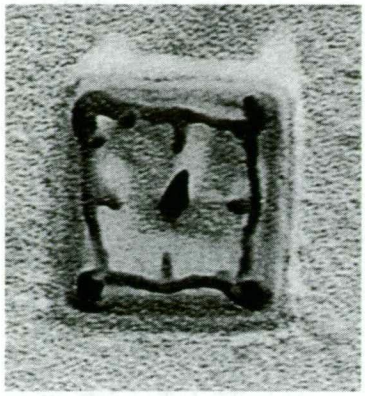


Fig. 5.41: *Pyramimonas* sp. 6, box scale (0.24 μm) with central spine; from Honey Moon Bay

(Micrograph no: 5375)

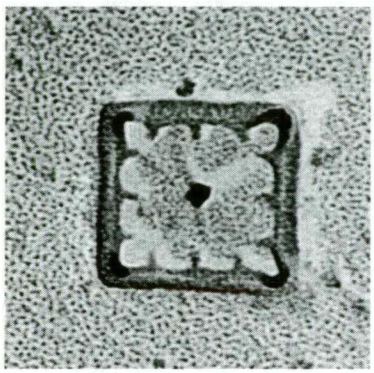


Fig. 5.42: *Pyramimonas* sp. 7, box scale (0.24 μm) with short central knob and perforated rim; from Fleurty Point

(Micrograph no: 5132)

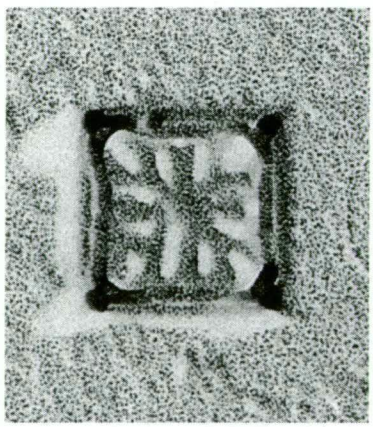


Fig. 5.43: *Pyramimonas* sp. 8, box scale (0.2 μm) with perforated pattern; from Honey Moon Bay

(Micrograph no: 5356)

5.4 Discussion

The most common prasinophyte observed in this survey was *Pyramimonas grossii*, found at 12 of the 21 sites. It also grew in enrichment culture, and a unialgal isolate is currently maintained in the CSIRO Collection of Living Microalgae (CS-489).

Generally, prasinophytes did not grow well in enrichment culture, with only *Mamiella gilva*, *Pyramimonas cirolanae* and *P. grossii* being successful in the growth media used. *P. grossii* tolerated a wide nutrient and salinity range (28 and 35 psu), whereas *M. gilva* and *P. cirolanae* only grew in higher nutrient and lower salinity media. *P. cirolanae* was common in samples from the Derwent River and Pipeclay Lagoon, but was not successfully maintained in culture. McFadden et al (1986) used the low nutrient medium, MET 44 (Schoene and Schoene, 1982), for successful enrichment and isolation of *Pyramimonas* species, and G/2 without soil extract was used for culture maintenance.

Of the number of marine prasinophytes previously described (Table 5.1), only a small percentage was identified in this survey. This may have been due to the fact that the majority of samples were shadow cast rather than stained with uranyl acetate, the latter being a far more suitable preparation technique to show the fine structure of these scales. In fact, identification of *Pyramimonas* species in the present study was usually made on the basis of larger scale structures, for example, crown, basket and box scales, rather than the smaller limuloid scales or flagellar hairs. Marin and Melkonian (1994) outlined a staining technique (2% aqueous uranyl acetate for 90 s and washing once with distilled water) to show the structure of flagellar hairs using cells were taken directly from young cultures, without previous centrifuging or filtering, as these procedures may result in excessive loss of flagellar hairs .

Scale size and structure is well conserved within prasinophyte species, and it has been shown that even differences in the flagellar hair scale structure can be used to separate species (Marin and Melkonian, 1994). Recent genetic studies of the phylogeny of *Pyramimonas* confirmed the taxonomical groupings based on scale morphology, internal ultrastructure and biochemical features (Daugbjerb et al, 1994). It is possible that species such as *P. amylifera* and *P. virginica*, with reported different biogeographical forms, may well comprise more than one species, and genetic analysis of these strains will be needed to resolve this issue.

Pyramimonas represents one of the largest and most morphologically diverse genera within the Prasinophyceae, with over 30 species studied by electron microscopy, and another 13 species known from light microscopical observations. Sixteen species of this genus have been described from Australian waters, and six known species were found in this survey, with a further eight previously undescribed species being illustrated.

Blooms of *Pyamimonas* are common in estuarine and coastal areas world-wide (Inouye et al, 1984), but have not had any reported harmful effects. In fact, no toxic prasinophytes have yet been identified, although a *Pyramimonas* isolate from Western Australia was recently found to be toxic to *Artemia* nauplii (pers. observation), and this requires further investigation. In contrast, *P. grossii* (CS-489) did not cause any mortalities when fed to *Artemia* nauplii (see Chapter 7).

This survey has shown that prasinophytes are commonly found in Tasmanian coastal waters, but additional work on the culture and identification of these organisms is required.

6. DIVISION DINOPHYTA

6.1 Scale-bearing Dinoflagellates

Only a few dinoflagellate species have scales, and very few dinoflagellates are less than 20 µm in size, hence they are only mentioned briefly here.

Scale-bearing dinoflagellates include: *Heterocapsa* species, which have small body scales, the morphology of which is used to differentiate between species (Hansen, 1995): *Oxyrrhis marina,* which has both flagellar and body scales (Clarke and Pennick, 1972, 1976); and *Lepidodinium viride*, a green dinoflagellate with a chorophyll-containing endosymbiont, and having basket-shaped scales covering the entire cell surface (Watanabe et al, 1990).

In this survey, one scale-bearing dinoflagellate, *Heterocapsa rotundata,* was found, and another possible dinoflagellate scale type illustrated.

6.2 Species Descriptions

***Heterocapsa rotundata* (Lohmann) Hansen** **Fig. 6.1**

As a small dinoflagellate, this species has caused various taxonomic problems and has been reclassified and renamed several times (Dodge, 1982; Hansen, 1995)

.

Synonyms: ***Amphidinium rotundatum*, Lohmann 1908**
***Gymnodinium minutum*, Lebour 1925**
***Massartia rotundatum* (Lohmann) Schiller 1933**
***Amphidinium pellucidum*, Redeke 1935**
***Amphidinium redekei*, Conrad et Kufferath, 1954**
***Katodinium rotundatum*, (Lohmann) Loeblich 1965**
***Katodinium minutum*, Sournia, 1973**

Micrographs: Hansen, 1989; Figs. 6 - 11.
Hansen, 1995; Figs. 2, 4 .

Present Findings.
Scales were found in samples from Dru Point and Deep Bay.

This species grew well in enrichment culture, particularly at lower nutrient concentrations, namely GSe/2; GSe/10 and modified L medium, in addition to GSe. Whole cells and scales were found in cultures established from Derwent River and Dru Point samples.

H. rotundata was successfully isolated by micromanipulation from a Derwent River GSe/2 enrichment culture. A clonal unialgal culture (CS-484) is currently maintained in the CSIRO Microalgae Culture Collection (in GSe medium at 15 °C, under standard growth conditions).

This is the first conclusive report of this species, based on scale fine-structural detail, from Australian coastal waters.

Description.

H. rotudata is an "amoured" dinoflagellate with thin thecal plates and an outer layer of roughly triangular scales (Hansen, 1989). Scales ranged from 0.32 - 0.46 μm ($\bar{x}$=0.36 μm; n=21) in length, and from 0.32 - 0.48 μm ($\bar{x}$=0.40 μm; n=21) in width. Each scale had one central spine, about 0.07um length (n=5), and nine peripheral shorter spines in groups of three at the triangle apices (Fig. 6.1). The base plate had a central ring and three pairs of distinctly-arranged radiating ribs, similar in shape to a radioactive symbol. This description agreed with that of Hansen (1989, Fig. 6) although scale sizes were slightly smaller.

Another scale type with a triangular shape and a similar size was found in a Pipeclay Lagoon sample (Fig. 6.2). This scale type resembled that of *Heterocapsa*, but did not match any of the ten known species (Morril and Loeblich, 1981; Hansen, 1995; Horiguchi, 1995, 1997).

Distribution.

Originally described from plankton in the Kiel Bight (Denmark), *H. rotundata* has been since reported as *K. rotundatum* from temperate coastal waters of Denmark, the UK, Belgium, Norway, Yugoslavia and eastern USA (Dodge 1982; Hansen, 1989; Jochem 1990). In 1991, a bloom of *H. rotundatum,* reaching over one million cells L^{-1}, occurred in New Bedford Harbour, USA (Borkman et al, 19993), but no harmful effects were recorded.

Heterocapsa species have also been reported from the central North Pacific Ocean (Hoepffner and Haas, 1990).

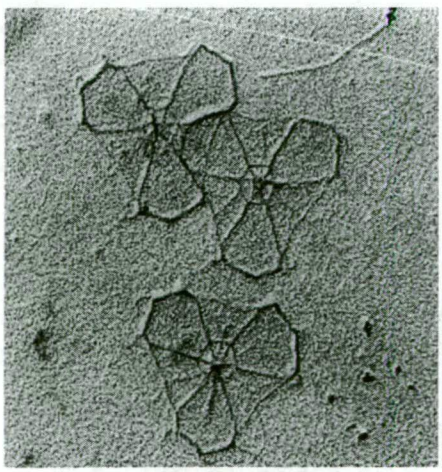


Fig. 6.1: *Heterocapsa rotundata* scales (c. 0.4 μm); from Dru Point

(Micrograph no: 4882)

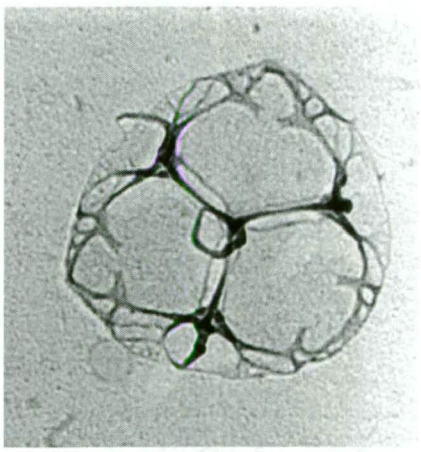


Fig. 6.2: *Heterocapsa*-like scale (c. 0.4 μm); from Pipeclay Lagoon

(Micrograph no: 5450)

7. TOXICITY OF SCALE-BEARING NANOFLAGELLATES TESTED USING LARVAL BRINE SHRIMP BIOASSAYS

7.1 Introduction

Potentially toxic nanoflagellates occur world-wide. Harmful algal blooms of the prymnesiophytes *Chrysochromulina* spp., *Prymnesium* spp. and *Phaeocystis* spp., as well as the scale-bearing dinoflagellate, *Heterocapsa circularisquama*, have had devasting effects on marine environments, as well as on farmed fish and bivalves, resulting in enormous economic loss (Edvardsen and Paasche, 1998; Honjo et al, 1998). To date, there has been no evidence of toxicity reported for marine chrysophyte or prasinophyte nanoflagellates.

Methods used to test for toxic effects of these nanoflagellates have included: the larval brine shrimp (*Artemia*) bioassay (Edvardsen, 1993; Larsen et al, 1993; Rhodes and Burke, 1996; Simonsen and Moestrup, 1997); feeding to other test organisms, for example, ciliates, copepods, bivalves and fish (Chang, 1985; Moestrup, 1994 and references therein; Nejstgaard and Solberg, 1996; Matsuyama et al, 1996; Kamiyama and Arima, 1997; Johansson, 1999); and examining algal extracts for effects on erythrocytes (fish, mouse, horse and human) and nerve cell preparations (Edvardsen et al, 1990; Yasumoto et al, 1990; Meldahl et al, 1995; Simonsen and Moestrup, 1997).

In this study, the *Artemia* bioassay was used to test nanoflagellate strains isolated from Tasmanian waters. This method has several advantages: the *Artemia* nauplii required for the bioassay are easily cultured; they are non-selective filter feeders, eating algal cells directly, thus eliminating the need for preparation of algal extracts; and they are used as a standard test species in bioassays (Persoone and Wells, 1987). However, *Artemia* is only found in nature at high salinities, for example, in salt lakes, and is therefore an artificial test organism. Nanoflagellate strains found to be toxic to *Artemia* nauplii were also tested for toxicity to local decapod larvae and hatchery-reared *Crassostrea gigas* oyster larvae.

Toxic activity in species of *Chrysochromulina* and *Prymnesium* has been shown to be related to conditions of increased physiological stress, and thus varies with culture age and growth parameters. For example, old cultures of *C. polylepis* were reported to be toxic to the bryozoan, *Electra pilosa*, whereas young cultures were an excellent food source (Jebram, cited in Moestrup 1994). Cultures of *C. polylepis*, *P. parvum* and *P. patelliferum* showed increased toxicity when grown under phosphorus or

nitrogen limitation (Edvardsen et al, 1990; Larsen et al, 1993; Simonsen and Moestrup, 1997; Johansson and Granéli, 1998; Johansson, 1999). In the present study, the effects of culture age and phosphorus depletion on the toxic activity of *P. patelliferum* strains isolated from Pipeclay Lagoon were examined and compared with literature findings.

7.2 Methods

7.2.1 Algal cultures

Twenty-six nanoflagellate strains from four different classes were tested for toxicity using *Artemia* bioassays. Twenty-three strains were isolated from Tasmanian waters (Table 7.1), with three strains of *Prymnesium patelliferum* obtained from mainland Australia and overseas (Table 7.2).

Cultures were grown in 40 mL GSe media in 50 mL Kimax® tool-rimmed Erlenmeyer flasks at 15, 18 or 20°C (Tables 7.1, 7.2). Light intensity was measured using a QSL-100 light meter (Biospherical Instruments), and was initially 80 - 100 µmol photons PAR $m^{-2} s^{-1}$, reduced to approximately 20 µmol photons PAR $m^{-2} s^{-1}$ after 7 - 10 days, in accordance with standard culturing practices of the CSIRO Collection of Living Microalgae (Jeffrey and LeRoi, 1997). Photoperiod was 12:12 h light:dark cycles.

Cultures were in stationary phase when tested (unless otherwise stated). Cells were counted using a Neubauer haemacytometer (Bright Line) to determine average cell density (Guillard, 1978; Throndsen, 1995).

7.2.2 *Artemia* bioassays

The *Artemia* bioassay involves feeding nauplii with a particular algal species, recording mortalities after 24 hours, and then calculating either LC_{50} (the cell concentration at which half the nauplii die), LD_{50} (the time for half the nauplii to die), or the overall percentage mortality (Persoone and Wells, 1987; Rhodes et al, 1994; Demaret et al, 1995). In this study, percentage mortality was recorded.

Artemia nauplii were hatched from cysts (San Francisco Bay, USA) and incubated for a total time of 48 hours before being used in bioassays. Cysts (1 g) were hatched in 500 mL filtered seawater (35 psu) at 28 - 30°C, under continuous illumination (80 - 100 µmol photons PAR $m^{-2} s^{-1}$), and using gentle aeration (Plankto and O'Sullivan, 1993; Hoff and Snell, 1987).

Hatched nauplii were checked microscopically for health and growth stage (instar II and III) and transferred, by careful pipetting, to 35 mm petri dishes (10 animals per petri dish). Algal cultures (2 mL) were then added to each petri dish, and all tests were carried out in quadruplicate. A GSe medium control (2 mL) was also included. Bioassays were held at standard algal growth conditions, and checked at regular intervals (1, 2, 4, 8 and 24 hours) to observe general health of the nauplii. Death was defined as non-motility for more than 10 seconds, combined with no response when the surrounding liquid was gently swirled.

7.2.3 Range finding tests

Strains of *Prymnesium patelliferum* (CS-376/3A, 3B, 3C), shown to be toxic to *Artemia* nauplii, were tested further to ascertain the effect of culture age and phosphorus depletion on toxic activity. A range finding test determines the "critical range" within which mortality changes from 0% at a low concentration to 100% at a high concentration.

Ten-fold dilutions of *P. patelliferum* cultures in logarithmic or stationary phase, grown in GSe or GSe-PO_4 media, were prepared using the respective medium as the dilutant. Two mL of each dilution was added to a 35 mm petri dish containing 10 *Artemia* nauplii, and bioassays were carried out as previously described. Medium controls (2 mL) were also included.

7.2.4 Cell-free filtrate preparation and testing

To determine if toxins were released into the surrounding medium, a cell-free filtrate of *Prymnesium patelliferum* CS-376/A was prepared and tested for toxicity to *Artemia* nauplii.

Twenty mL of *P. patelliferum* culture (grown in GSe medium under standard conditions) was gently gravity-screened through 5 µm plankton mesh, and then filtered through 0.45 µm and 0.22 µm filter-membranes under aseptic conditions. Two mL of this final filtrate was added to each of four 35 mm petri dishes containing 10 *Artemia* nauplii, and bioassays were carried out as previously described. A GSe medium control (2 mL) was also included.

7.2.5 Oyster and decapod larval bioassays

Hatchery-reared *Crassostrea gigas* larvae (c. 150 µm; 10 days old) were supplied by Shellfish Culture Pty Ltd. Decapod larvae (c. 100 µm; a zoea stage) were collected from the Derwent River (CSIRO) using a 200 µm mesh plankton net (25 m horizontal tow).

Two algal strains were tested: *Prymnesium patelliferum* CS-376/3A and *Pavlova pinguis* CS-375/1. Cultures were grown in GSe medium under standard conditions, and were in late logarithmic to early stationary phase at the time of testing.

Larvae were transferred, by pipetting, to 35 mm petri dishes (5 animals per petri dish). Algal cultures (2 mL) were then added. To ensure larvae would survive under the test conditions, oyster larvae were placed into 2 mL of filtered seawater, and decapod larvae into 2 mL of Derwent River water. A GSe medium control (2 mL) was also included. Bioassays were held at standard algal growth conditions, and checked at 24 and 48 hours to record larval health. Death was defined as non-motility for more than 10 seconds, combined with no response when the surrounding liquid was gently swirled.

Table 7.1: Nanoflagellate strains from Tasmanian waters tested for toxicity using *Artemia* bioassays.

STRAIN	CS- CODE	LOCALITY	DATE ISOLATED	GROWTH TEMP.
CHRYSOPHYTA				
Chrysophyceae				
Chrysolepidomonas cf. *marina*	CS-490	Derwent River	January, 1995	18°C
Prymnesiophyceae				
Chrysochromulina acantha	CS-480	Derwent River	July, 1994	15°C
Chrysochromulina apheles	CS-481	Derwent River	July, 1994	20°C
Chrysochromulina hirta	CS-482	Derwent River	October, 1994	15°C
Chrysochromulina aff. *scutellum*	CS-496	Derwent River	June, 1994	15°C
Chrysochromulina simplex	CS-483	Derwent River	June, 1994	20°C
Chrysochromulina sp. ("eyelash")	CS-410/8	Derwent River	July, 1994	15°C
Chrysochromulina sp. ("eyelash")	CS-410/11	Derwent River	July, 1994	20°C
Pavlova pinguis	CS-375/1	Pipeclay Lagoon	June, 1992	18°C
Pavlova pinguis	CS-375/3	Pipeclay Lagoon	June, 1992	18°C
Pavlova pinguis	CS-375/5	Pipeclay Lagoon	June, 1992	18°C
Pavlova sp.	CS-491	Pipeclay Lagoon	December, 1992	15°C
Pavlova sp.	CS-492/8	Pipeclay Lagoon	June, 1996	15°C
Pavlova sp.	CS-492/3	Pipeclay Lagoon	June, 1996	15°C
Phaeocystis globosa	CS-495	Pirates Bay	January, 1995	10°C
Prymnesium patelliferum	CS-376/3A	Pipeclay Lagoon	January, 1993	18°C
Prymnesium patelliferum	CS-376/3B	Pipeclay Lagoon	January, 1993	18°C
Prymnesium patelliferum	CS-376/3C	Pipeclay Lagoon	January, 1993	18°C
Prymnesium sp.	CS-494	Derwent River	April, 1995	15°C
Prymnesium sp.	CS-493/1	Pipeclay Lagoon	June, 1996	18°C
Prymnesium sp.	CS-493/4	Pipeclay Lagoon	June, 1996	20°C
CHLOROPHYTA				
Prasinophyceae				
Pyramimonas grossii	CS-489	Derwent River	November, 1994	15°C
DINOPHYTA				
Dinophyceae				
Heterocapsa rotundata	CS-484	Derwent River	July, 1994	15°C

Table 7.2: *Prymnesium* strains from Australia and overseas tested for toxicity using *Artemia* bioassays.

STRAIN	CS-CODE	LOCALITY	DATE	GROWTH TEMP.
Prymnesium sp.	CS-458	Serpentine River, Western Australia	Isolated 1992 (J.M. LeRoi)	18°C
Prymnesium patelliferum	CS-288 (PCC 527) [1]	The Fleet, Dorset, United Kingdom	Received 1992	18°C
Prymnesium patelliferum	CS-345 (CAWP 12) [2]	New Zealand	Received 1995	18°C

[1] PCC = Plymouth Culture Collection, Plymouth, UK.

[2] CAWP = Cawthron Microalgae Culture Collection, Nelson, NZ.

7.3 Results

7.3.1 *Artemia* bioassays

Percentage mortalities after 24 hours are recorded in Table 7.3. Most strains tested were not toxic to *Artemia* nauplii. However, Tasmanian strains of *Prymnesium patelliferum* and *Prymnesium* spp. were toxic, with over 50% of nauplii dying in the first eight hours (Fig. 7.1). *Prymnesium patelliferum* strains from New Zealand (CS-345) and the UK (CS-288) caused fewer mortalities than the Tasmanian strains, as did the *Prymnesium* strain from Western Australia (CS-458). *Heterocapsa rotundata* was the only other strain to cause mortality. Nauplii fed *C. acantha* and *C.* aff. *scutellum* markedly slowed their swimming, but did not die during the 24 hour test period.

In contrast, some strains supported growth of *Artemia* nauplii to adults after several days; these included *Pavlova pinguis*, *Chrysochromulina* sp. ("eyelash") and *Pyraminonas grossii.*

No detrimental effects were observed for the *Artemia* nauplii in the GSe medium controls.

7.3.2 Effects of culture age and phosphorus depletion on *Prymnesium patelliferum* toxicity

Percentage mortalities for *Artemia* nauplii exposed to different concentrations of *P. patelliferum* cultures (CS-376/3B) at different growth phases are given in Table 7.4. Logarithmic phase cultures were more toxic than stationary phase cultures at cell concentrations $<10^2$ cells mL^{-1}.

Percentage mortalities for *Artemia* nauplii exposed to different concentrations of *P. patelliferum* cultures (CS-376/3A, 3B and 3C) in logarithmic phase, grown in GSe and GSe-PO_4 media, are given in Tables 7.5 and 7.6. Cultures grown under phosphorus depletion were more toxic at the low cell concentration of 10^1 cells mL^{-1}, in comparison with cultures grown in phosphorous replete medium. However, *P. patelliferum* CS-376/3B, when grown under phophorus depletion, was more toxic than the other two strains at cell concentrations $<10^3$ cells mL^{-1}.

Percentage mortalities for *Artemia* nauplii exposed to different concentrations of *P. patelliferum* cultures (CS-376/3A, 3B and 3C) in stationary phase, grown in GSe and GSe-PO_4 media, are given in Tables 7.7 and 7.8. These bioassays were done some time after the previous bioassays and results were not directly comparable.

Cultures grown under phosphorus depletion had lower initial cell densities and were more toxic at cell concentrations $<10^3$ cells mL^{-1}. In this set of bioassays, different results were obtained for the three *P. patelliferum* strains: CS-376/3C was non-toxic when grown in GSe medium but toxic when grown under phophorus depletion, and CS-376/3B was again more toxic than the other two strains.

No detrimental effects were observed for the *Artemia* nauplii in the medium controls.

7.3.3 Effect of the cell-free filtrate of *Prymnesium patelliferum* on *Artemia* nauplii

No mortalities were recorded, nor were any detrimental effects observed, for the *Artemia* nauplii exposed to the cell-free filtrate of *P. patelliferum.*

7.3.4 Oyster and decapod larval bioassays

The hatchery-reared oyster larvae fed *Prymnesium patelliferum* (CS-376/3A) stopped swimming after 24 hours, whereas those fed *Pavlova pinguis* (CS-375/1), and those in the filtered seawater, continued to actively swim.

Decapod zoea, when fed *Prymnesium patelliferum*, stopped swimming and instead exhibited "twitching" after 48 hours. Zoea fed *Pavlova pinguis* continued to actively swim, as did the zoea in the Derwent River water. As these zoea were at a very early developmental stage, they lacked species-specific features and were unable to be identified further (R. King, pers. comm.).

No detrimental effects were observed for either larval species in the GSe medium controls.

Fig. 7.1: Percentage mortalities of *Artemia* nauplii over 24 hours when fed different strains of *P. patelliferum*

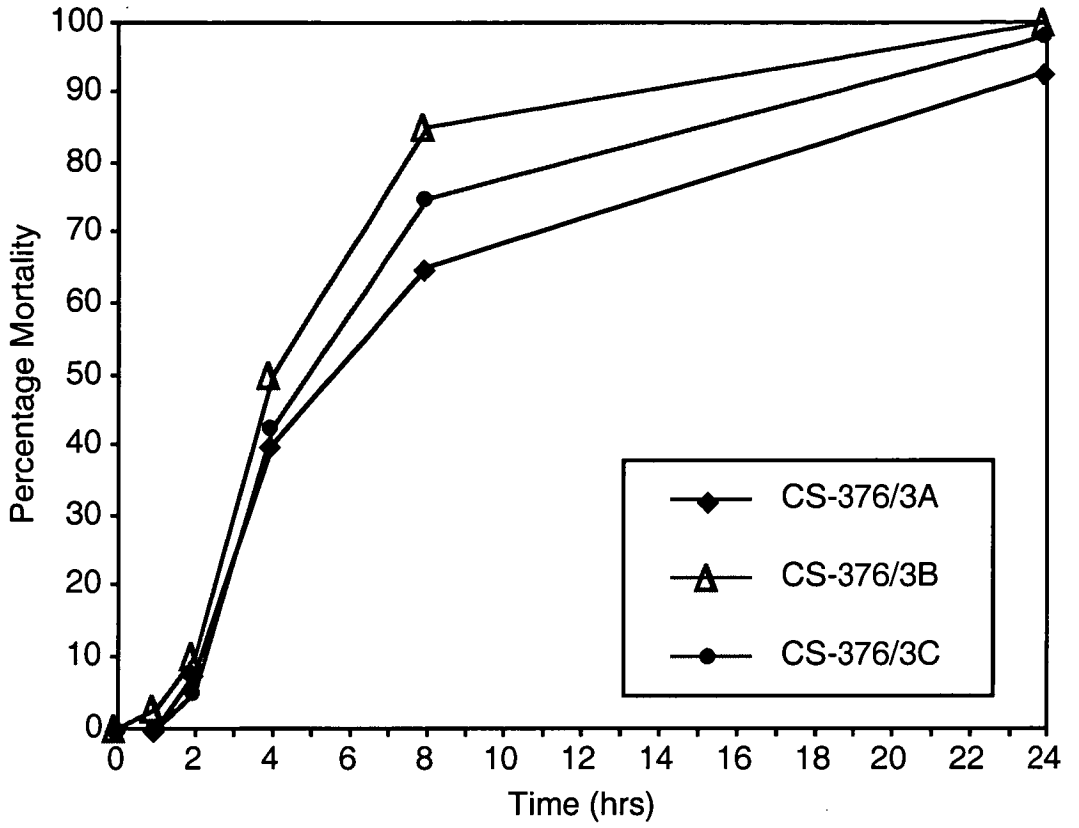


Table 7.3: Percentage mortalities of *Artemia* nauplii after 24 hours when fed different nanoflagellate strains.

STRAIN	CS-CODE	CELL DENSITY ($x\ 10^6$ cells mL^{-1})	PERCENTAGE MORTALITY
CHRYSOPHYTA			
Chrysophyceae			
Chrysolepidomonas cf. *marina*	CS-490	1.18	0
Prymnesiophyceae			
Chrysochromulina acantha	CS-480	0.38	0 (1)
Chrysochromulina apheles	CS-481	4.20	0
Chrysochromulina hirta	CS-482	0.90	0
Chrysochromulina aff. *scutellum*	CS-496	0.66	0 (1)
Chrysochromulina simplex	CS-483	1.18	0
Chrysochromulina sp. ("eyelash")	CS-410/8	1.70	0
Chrysochromulina sp. ("eyelash")	CS-410/11	1.25	0
Pavlova pinguis	CS-375/1	7.20	0
Pavlova pinguis	CS-375/3	4.04	0
Pavlova pinguis	CS-375/5	4.55	0
Pavlova sp.	CS-491	5.60	0
Pavlova sp.	CS-492/8	1.48	0
Pavlova sp.	CS-492/3	1.20	0
Phaeocystis globosa	CS-495	12.8	0
Prymnesium patelliferum	CS-376/3A	3.01	100
Prymnesium patelliferum	CS-376/3B	3.04	100
Prymnesium patelliferum	CS-376/3C	4.98	100
Prymnesium sp.	CS-494	2.65	95
Prymnesium sp.	CS-493/1	5.70	93
Prymnesium sp.	CS-493/4	2.84	100
Prymnesium sp. (WA)	CS-458	4.73	15
Prymnesium patelliferum (UK)	CS-288	0.95	25
Prymnesium patelliferum (NZ)	CS-345	1.10	60
CHLOROPHYTA			
Prasinophyceae			
Pyramimonas grossii	CS-489	4.38	0
DINOPHYTA			
Dinophyceae			
Heterocapsa rotundata	CS-484	1.10	12.5

(1) Although *Artemia* nauplii were all alive, they showed a marked decrease in motility.

Table 7.4: Percentage mortalities of *Artemia* nauplii exposed for 24 hours to different concentrations of *Prymnesium patelliferum* cultures (CS-376/3B) at different growth phases.

GROWTH PHASE	CELL DENSITY RANGE (cells mL^{-1})						
	10^6	10^5	10^4	10^3	10^2	10^1	1
Logarithmic	100	100	100	100	100	10	0
Stationary	100	100	100	100	35	0	0

Table 7.5: Percentage mortalities of *Artemia* nauplii exposed for 24 hours to different concentrations of *Prymnesium patelliferum* cultures (logarithmic phase) grown in GSe medium.

STRAIN	CELL DENSITY RANGE (cells mL^{-1})						
	10^6	10^5	10^4	10^3	10^2	10^1	1
CS-376/3A	100	90	90	90	80	10	0
CS-376/3B	100	100	100	100	100	10	0
CS-376/3C	100	100	100	100	80	10	0

Table 7.6: Percentage mortalities of *Artemia* nauplii exposed for 24 hours to different concentrations of *Prymnesium patelliferum* cultures (logarithmic phase) grown in GSe-PO_4 medium.

STRAIN	CELL DENSITY RANGE (cells mL^{-1})						
	10^6	10^5	10^4	10^3	10^2	10^1	1
CS-376/3A	100	100	90	80	30	30	0
CS-376/3B	100	100	100	100	90	40	0
CS-376/3C	100	100	90	80	60	40	0

Table 7.7: Percentage mortalities of *Artemia* nauplii exposed for 24 hours to different concentrations of *Prymnesium patelliferum* cultures (stationary phase) grown in GSe medium.

STRAIN	CELL DENSITY RANGE (cells mL^{-1})						
	10^6	10^5	10^4	10^3	10^2	10^1	1
CS-376/3A	100	70	70	10	0	0	0
CS-376/3B	90	70	70	30	0	0	0
CS-376/3C	0	0	0	0	0	0	0

Table 7.8: Percentage mortalities for *Artemia* nauplii exposed for 24 hours to different concentrations of *Prymnesium patelliferum* cultures (stationary phase) grown in GSe-PO_4 medium.

STRAIN	CELL DENSITY RANGE (cells mL^{-1})					
	10^5	10^4	10^3	10^2	10^1	1
CS-376/3A	90	70	70	50	0	0
CS-376/3B	80	80	70	80	20	10
CS-376/3C	70	40	40	20	0	0

7.4 Discussion

Of the twenty-six nanoflagellate strains from four different classes tested in this study, only *Prymnesium* species were found to be toxic to larval brine shrimp nauplii. Both *Prymnesium parvum* and *P. patelliferum* are already known to be toxic to *Artemia* nauplii, although toxicity of different strains may vary, even if grown under the same conditions (Larsen et al, 1993; Meldahl et al, 1994).

None of the *Chrysochromulina* strains tested in the present study were found to be toxic, which agreed with the results of previous *Artemia* bioassays using overseas *Chrysochromulina* cultures (Edvardsen, 1993; Rhodes et al, 1996; Simonsen and Moestrup, 1997). This was the first time that *Chrysochromulina* sp. ("eyelash") had been tested for toxicity.

The inhibitory effect of *Chrysochromulina acantha* and C. aff. *scutellum* on nauplii swimming observed in this study has not been previously reported. *C. acantha* was found to be non-toxic to *Artemia* nauplii (Edvardsen and Paasche, 1992), and *C. scutellum* was also reported to be non-toxic in two separate bioassays (Edvardsen, 1993; Eikrem and Moestrup, 1998). In the latter case, it is possible that the Tasmanian strain is a different species to *C. scutellum*, (as not all scale types characteristic of this species were found in Tasmanian samples), or alternatively, it may be a strain which does produce inhibitory substances. These results call for further investigation.

Chrysochromulina hirta, with its array of large spine scales, did not have any effect on *Artemia* nauplii. *C. hirta* was one of three spine-bearing *Chrysochromulina* species implicated in a fish-killing bloom in Danish coastal waters (Knipschildt, 1992; Hansen et al, 1995), and gill damage by these spines may have contributed to fish mortality.

The only other species which had detrimental effects on *Artemia* nauplii was *Heterocapsa rotundata*. Although percentage mortality was low (12.5%), this species should be regarded as potentially toxic and tested using other organisms. Another *Heterocapsa* species, *H. circularisquama*, is known to have lethal and sublethal effects on bivalves, including pearl oysters, Pacific oysters and short-necked clams (Honjo et al, 1998), and tintinnid ciliates (Kamiyama and Arima, 1997).

Phaeocystis globosa was found to be non-toxic to *Artemia* nauplii in this study, but *P. pouchetii* has been recently found to show toxicity towards fish larvae, and appears to contain toxic substances that differ in their mode of action from other prymnesiophytes, for example, in having anaesthetic effects (Stabell et al, 1999).

A single species bioassay has obvious limitations when testing for toxicity, and in this study, hatchery-reared oyster larvae and local Derwent River decapod zoea were also used as bioassay organisms. *Prymnesium patelliferum* had detrimental effects on both these larval types. *P. patelliferum* has also been reported to supress copepod feeding and fecundity (Nejstgaard and Solberg, 1996), and *P. parvum* has been shown to have lethal effects on the ciliate, *Euplotes* (Johansson, 1999).

Given that *Prymnesium patelliferum* is toxic to *Crassostrea gigas* oyster larvae, it would be useful to study the effects of this species on the oyster spat. *P. patelliferum* was isolated from Pipeclay Lagoon, which is the site of a major oyster nursery, as well as a oyster outgrowing area. In the nursery, spat are held in upwellers, through which seawater is pumped providing the animals with food particles, including naturally-ocurring algae. As seen in the present study, *P. patelliferum* has detrimental effects even at low concentrations, and its presence in seawater may affect spat growth rates, already known to be slow during the winter months.

Previous studies have shown that nutrient limitation enhances toxicity (Johansson and Granéli, 1998, 1999a, b). Consequently, cultures in stationary phase would be expected to be more toxic than those in logarithmic phase. However, *P. patelliferum* CS-376/3B was found to be more toxic to *Artemia* nauplii when in logarithmic phase (Table 7.4). Cultures used in this bioassay were non-axenic, and it may have been possible that bacteria, usually present in higher numbers in stationary phase cultures than logarithmic ones, were supplying nutrients in the stationary phase culture. Johansson (1999) and co-workers showed that phosphorus from ingested bacteria decreased toxicity of *P. patelliferum.*

In comparison, Rhodes (1994) found no change in toxicity of *P. parvum* regardless of growth phase. Cultures at 2, 4, 6, 8, 10 or 50 days all gave the same result when tested with *Artemia* nauplii. Axenic cultures were used in this study.

Phosphorus depletion was clearly seen to enhance toxicity of *P. patelliferum* in both logarithmic and stationary phase cultures (Tables 7.5 - 7.8), in agreement with findings by Larsen et al (1993) who examined both Norwegian and Australian

strains. Johansson and Granéli (1998) reported that the highest toxicity was found in *P. parvum* cultures when grown under nitrogen limitation, and in *C. polylepis* cultures when grown under phosphorus limitation. In the present study, stationary phase cultures of *P. patelliferum* CS-376/3C were non-toxic when grown in GSe medium, but toxic when grown under phosphorus deplete conditions. This has important implications in that potentially toxic species may become toxic under certain environmental conditions.

The toxicity of nanoflagellate species demonstrated during blooms is not always reproducible under laboratory conditions. During the 1998 *C. polylepis* bloom, a range of marine organisms were killed, but the effects of *C. polylepis* cultures, established from the bloom, on test organisms in the laboratory were not dramatic (Edvardsen and Paasche, 1998, and references therein). Similarly, water samples containing *C. leadbeateri* from the 1991 fish-killing bloom were toxic to *Artemia* nauplii and to nerve cell preparations, but cultures established from these samples were non-toxic (Edvardsen, 1993; Meldahl et al, 1994). Removing cells from the variety of biotic interactions that occur in the natural environment apparently has an effect on toxin production in culture. In addition, under laboratory growth conditions, nutrients in culture media are usually far in excess of those concentrations found in natural seawater, and consequently, lacking conditions of physiological stress, cells may not produce toxins.

The time over which a species is cultured also appears to have an effect on toxicity. *C. polylepis* cultures retained their toxicity to *Artemia* nauplii even after five years in culture (Edvardsen and Paasche, 1992; Edvardsen, 1993), whereas *P. parvum* loses toxicity with time in culture. However, when grown in phosphorus deplete medium, it quickly regains toxicity (L. Rhodes, pers. comm.). *P. patelliferum* CS-376/3A, 3B and 3C strains were in culture for at least three years before being tested for toxicity to *Artemia* nauplii, and after five years in culture, were still toxic, with the exception of CS-376/3C. This strain only showed toxicity when grown under phosphorus deplete conditions. These three *P. patelliferum* strains were isolated from the same water sample and maintained under identical culture conditions, and yet they have different toxic activity.

Prymnesium parvum, P. patelliferum and *P. calathiferum* are known to excrete toxic substances with negative effects on the growth of different species of microalgae as well as fish (Moestrup, 1994; Rhodes, 1994; Johansson and Granéli, 1999a). However, the cell-free filtrate of *P. patelliferum* CS-376/3A had no effect on *Artemia*

nauplii. Excretion of these substances is strongly influenced by the availability of nutrients (Johansson, 1999), and thus further experiments are required to test effects on *Artemia* nauplii of cell-free filtrates from *P. patelliferum* cultures grown under nutrient deficient conditions.

Even though harmful algal blooms of *Prymnesium* species have not been reported for Tasmanian waters, this potentially toxic genus is present. Further studies on local *Prymnesium* strains to determine optimal growth temperatures and salinities, as well as toxin production under different environmental conditions, would be useful to assess the potential of this species to cause harmful blooms in Tasmanian coastal waters.

8. CONCLUSION

The present work has added considerably to our current knowledge of the nanoflagellate flora of southern Australian waters, with 17 new distribution records and over 70 species illustrated. Of these, the majority were from the Prymnesiophyceae (40 species), with 32 known *Chrysochromulina* species recorded. Thirteen prasinophytes were found, with the most common genus being *Pyramimonas* (7 species). Eleven scale-bearing marine chrysophytes were also recorded, *Paraphysomonas* being the most frequently observed genus (8 species). In addition, a scale-bearing dinoflagellate, *Heterocapsa rotundata*, was found, as well as three *Thaumatomastix* species and *Petasaria heterolepis*, these two latter genera being of uncertain taxonomic affiliation. This is the first time that scale-bearing nanoflagellates have been studied in Tasmanian waters and their biodiversity elucidated.

This survey has also provided a glimpse of yet undescribed taxa, with two new *Chrysochromulina* species able to be characterised, as well as two new *Paraphysomonas* species and three new *Pyramimonas* species being illustrated. In addition, a variety of new scales were seen, but a lack of complete cells meant that these species could not be fully described. Over 19 new *Chrysochromulina*-like scales were seen, in addition to five previously unreported *Pyramimonas* box scale types, and at least five forms of *Thaumatomastix* scale. These findings emphasise the considerable biodiversity of scale-bearing nanoflagellates in Tasmanian waters.

In this preliminary survey, the number of different species found in any one sample was often high. For example, over ten different species were often seen in a single water sample, especially from sheltered inshore areas. Samples collected from the D'Entrecastreaux area were high in nanoflagellate diversity, whereas samples from Pipeclay Lagoon often contained nanoplanktonic diatoms and coccolithophorids. The Derwent River was particularly rich in scale-bearing chrysophytes, especially *Paraphysomonas* spp as well as *Thaumatomastix*. Analyses of water quality parameters (in addition to temperature) associated with high nanoflagellate diversity would have been valuable.

In contrast, samples collected off-shore (Storm Bay, Maria Island and Pirates Bay) were often low in species diversity. Interestingly, this trend was reversed in enrichment culture, with a higher percentage of species from off-shore waters growing in culture in comparison with inshore waters.

These observations emphasise the need for seasonal studies. Very few seasonal comparisons of nanoflagellates have been done. A study by Smith and Hobson (1994) of a Canadian fjord showed that prymnesiophyte and prasinophyte species were dominant during summer (June - August) and chrysophyte species were common during winter (November to February). They also noted that *Apedinella spinifera* and *Phaeocystis pouchetii* were found throughout the year, with *Pyramimonas grossi* and *Meringosphaera mediterranea* present at most times of the year.

The present study has confirmed that many scale-bearing nanoflagellates reported from temperate coastal waters in the northern hemisphere are also found in southern hemisphere. *Chrysochromulina* species recently described from the northern hemisphere, for example *C. ahrengotii* (Jensen and Moestrup, 1999), *C. fragaria* (Eikrem and Edvardsen, 1999) and *C. scutellum* (Eikrem and Moestrup, 1998), had already been recorded from Australia (Beech, 1983; this study) as unidentified taxa. Only one species, *Chrysochromulina novae-zelandiae*, has been reported solely from the southern hemisphere.

The majority of studies on scale-bearing nanoflagellates to date have been carried out in temperate coastal waters, usually inspired by harmful algal blooms, such as the devasting 1988 *Chrysochromulina polylepis* bloom. In comparison, studies on these organisms from tropical areas, and from oceanic waters, are few.

Results of this survey have confirmed the presence and potential threat of toxic prymnesiophytes, including *C. polylepis* and *C. leadbeateri,* both harmful algal bloom-causing organisms in the northern hemisphere, and *Prymnesium patelliferum,* blooms of which are responsible for fish kills world-wide. Cultures of *P. patelliferum* from Pipeclay Lagoon were found to be toxic to *Artemia* nauplii as well as to hatchery-reared oyster larave and local Derwent River zooplankton (decapod zoea). *P. patelliferum* was only reported from two locations, Pipeclay Lagoon and Little Swanport, both of which are major areas for oyster grow-out and nursery rearing.

Another potentially toxic organism found in this survey was *Heterocapsa rotundata* which caused mortality in *Artemia* nauplii. Blooms of the related *Heterocapsa circularisquama* have resulted in mass mortality of bivalves in Japan (Horiguchi, 1995; Honjo et al, 1998).

These species add to the known list of problem microalgae in Tasmania, including the toxic PSP dinoflagellate, *Gymnodinium catenatum*, the domoic-acid producing *Pseudo-nitzschia australis*, the DSP dinoflagellate genus, *Dinophysis*, and the palytoxin-producing benthic dinoflagellate, *Ostreopsis*. Consequently, any bloom in Tasmanian waters should be investigated for possible toxic implications.

In contrast, *Pavlova pinguis,* isolated and identified in this study, is successfully used in Tasmanian hatcheries as a new food species for oyster larvae and spat. Used as a monospecific diet, it substantially increased growth of *Crassotrea gigas* oyster spat, and had a high nutritional quality and efficient ingestion rate (Brown et al, 1998). Like other *Pavlova* species, *P. pinguis* is a particularly rich source of polyunsaturated fatty acids (Brown et al, 1989; Jeffrey et al, 1994). Another potential food species was the prymnesiophyte, *Imantonia rotunda*, commonly found in water samples, which grew easily in culture and survived at low temperatures (<15 °C), thus making it suitable for outdoor pond culture. To date, no nutritional or feeding studies of this species have been undertaken.

Pyramimonas species have been successfully used in overseas aquaculture operations as live feeds for bivalve mollusc larvae (Coutteau, 1996), but are not often used in Australian hatcheries. Instead, the prasinophytes, *Tetraselmis suecica* and *T. chuii,* are common live feed species (Jeffrey et al, 1994). Both *Pyramimonas* and *Tetraselmis* species have a good overall nutritional quality, with respect to protein, lipid and carbohydrate (Brown and Jeffrey, 1992; Dunstan et al, 1992; Jeffrey et al, 1992). *Tetraselmis* species are easily grown in large-scale culture, whereas it is likely that, given some of the culturing difficulties experienced in the present study, *Pyramimonas* species would not grow well in mass quantities, thus limiting their potential use in aquaculture.

Further investigations required include fully characterising some of the many species found in this survey. Improved sampling and sample preparation protocols, which allow these small and often fragile cells to retain their scaly covering, are required. Techniques which enhance resolution of scale detail would also be advantageous in defining morphological features that differentiate species. The staining technique described by Marin and Melkonian (1994) for flagellar hairs allows study of ultrastructural detail not previously seen.

Although over 70 species were found in this survey, only 20 unialgal strains were successfully isolated and maintained in culture, indicating that there are a number of species with specific environmental growth requirements not met by standard laboratory culturing conditions. Culturing of strains allows a ready supply of material for further taxonomic, biochemical and physiological study. There is a need for new culturing procedures that allow species to be readily grown and easily maintained. While some species grow well in standard high nutrient enrichment media, for example, *Prymnesium patelliferum*, mixotrophic species may require higher levels of organic substances, and other species may prefer low nutrient concentrations. Even low nutrient media used in this study, such as modified L medium, had nutrient concentrations in excess of those found in natural waterways.

Scale morphology remains a useful criterion in the identification of prymnesiophytes, prasinophytes and certain chrysophytes, confirmed by recent genetic studies (Daugbjerg et al, 1994; Caron et al, 1999; Medlin et al, 2000). However, there is little taxonomic expertise in this field in Australia, and this study is only the third undertaken on marine scale-bearing nanoflagellates. Given that some of these species are toxic, there needs to be the expertise available to identify nanoflagellate blooms to species level, and to evaluate potential toxic implications.

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